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LABORATORY EXPERIMENTS
IN
PHYSIOLOGICAL CHEMISTRY

BOOKS BY

ARTHUR K. ANDERSON

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LABORATORY EXPERIMENTS
IN
PHYSIOLOGICAL CHEMISTRY

BY

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First Edition, Revised Printing

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PREFACE

I have written this laboratory outline to supply an adequate set of experiments for courses where my *Essentials of Physiological Chemistry* is the textbook. I hope that it may also find use in courses in connection with other textbooks.

This revised printing is quite similar to the first, but I have made several corrections and many minor changes that should make it more useful. For convenience, I have included several figures and tables from my textbook. A more satisfactory method is given for the preparation of a gold sol, and a new modification of the Kjeldahl method for determining nitrogen is presented. The methods for determining sugar in milk and blood have been simplified. The determination of the basal metabolic rate is discussed in more detail. Many of the directions for the preparation of reagents have been clarified. A new experiment is included on the composition of bone.

In this outline I have undoubtedly included more experiments than the average class can be expected to perform in six hours of laboratory work per week for one semester. Since the textbook is being used for various types of students, however, I believe that it is desirable to provide considerable leeway for the instructor to choose experiments especially suited to his needs.

I have assumed that many of the students using this book will have had no training in quantitative analysis; I have, therefore, included some elementary experiments designed to give the student sufficient training in volumetric analysis so that he will be able to conduct some of the more common procedures encountered in physiological chemistry. It has been my experience that some quantitative laboratory work in physiological chemistry adds a great deal to the interest of the course; it also stimulates accuracy in performing qualitative experiments.

For the convenience of those who are not interested in including quantitative experiments in their laboratory course, and for those desiring some of the simpler experiments for brief or very elementary courses, I have indicated, by starring (*) the number, those experiments that are quantitative in nature and a few of those that require more than ordinary techniques. The unstarred experiments can, I believe, be completed by an average class having from four to six laboratory hours available per week for one semester.

Much of the laboratory work in physiological chemistry is of necessity qualitative. In such experiments I have avoided anticipating the results to be expected. I have found that students lose interest in qualitative experiments when they know at the outset the results to be looked for.

After each experiment I have placed several questions which bring out the important points the experiment should teach. Many of the questions may be answered from observations made during the progress of the experiment; others are of a more general nature and will require a careful study of the textbook before answering. It has been my experience that the latter type of question aids materially in coordinating lecture and laboratory work. Students will find *Practical Physiological Chemistry* by Hawk, Oser and Summerson, published by the Blakiston Company of Philadelphia, an excellent reference book for finding the answers to some of the questions.

For the convenience of the instructor I have included in the Appendix a list of suggested desk reagents, a list of special apparatus necessary for conducting all the experiments given in this book, and also a list of chemicals, together with the approximate amount of each which should be ample for a class of twenty-five students.

ARTHUR K. ANDERSON

STATE COLLEGE, PENNSYLVANIA
September, 1946

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INTRODUCTION

Much of the work in a laboratory course in physiological chemistry is analytical. It therefore seems proper to discuss briefly some of the phases of analytical procedure most commonly encountered in biochemistry. Analytical chemistry may be divided into two types, namely, **qualitative analysis,** in which merely the presence of a certain substance is determined without regard to amount, and **quantitative analysis,** in which the exact amount of a given substance present is determined. Since quantitative analysis employs rather intricate procedures, it is necessary to discuss in detail some of the more important principles involved.

Analytical Balance. Since all quantitative chemical analyses ultimately depend upon the analytical balance, it is important at the outset that the student become familiar with this instrument. An analytical balance (see Fig. 1) is an extremely delicate instrument, and knowledge of how to use it is fundamental. It consists of a beam, at each end of which hang pans for holding weights and objects to be weighed. The beam is supported at its center by a post, on top of which is an agate plate. Built into the center of the beam is a knife-edge of agate. When the balance is in use, the agate knife-edge of the beam rests on the agate plate of the post. The pans are also supported by a similar combination of agate plates and knife-edges. When the balance is at rest, the knife-edges are not in contact with the agate plates, the beam and pan supports being elevated by a special supporting device. In order to prevent the knife-edges from becoming dulled they should be in contact with the agate plates as little as possible. Objects should never be placed on or removed from pans unless the balance is at rest and the beam and knife-edges are properly supported. The balance is enclosed within a glass case that prevents air currents from interfering with an accurate balance during weighing. Every balance case is supplied with a level, and it is very important that a balance be level when in use.

Balance cases are supported on adjustable legs to facilitate leveling.

Before weighing, it should always be ascertained that the balance is properly adjusted. This is done by carefully lowering the knife-edges onto their agate plates by means of the milled screw in the center of the base of the balance. The pan supports should then be lowered carefully by pressing the button at the left of the milled screw. The balance will start swinging, and the pointer should be watched closely. It is important to stand directly in front of the center of the scale behind the pointer. If the balance is in proper adjustment, the pointer should swing equal distances on each side of the center of the scale. The point at which the pointer would come to rest if allowed to do so is called the **zero point** of the balance. The method for determining the zero point of a balance is discussed below. If the pointer does not swing equal distances on each side of the center of the scale, adjustment should be made by turning the small screw at either end of the beam. This must be done with extreme care. As a rule a fraction of a turn on the screw is sufficient. Before

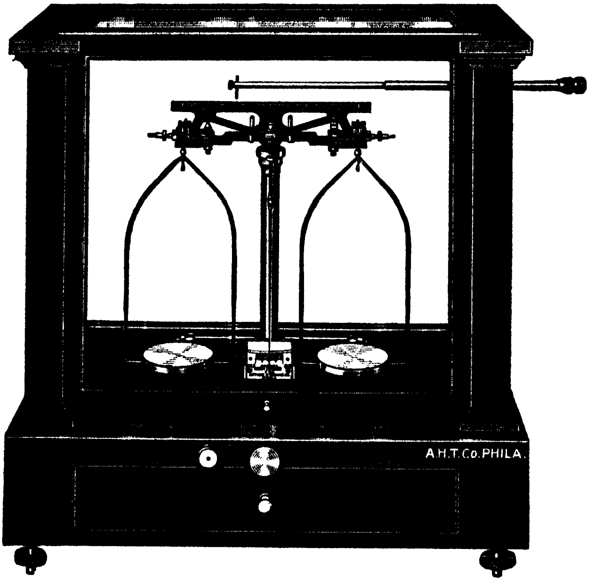

Fig. 1. Analytical balance.

making this adjustment, the balance must be brought to rest by supporting the beam and pans by turning the milled screw in the base of the balance back to its original position. In order to avoid jarring the knife-edges, the milled screw should be turned as the pointer is passing the center of the scale.

When the balance is in adjustment, it is ready for weighing. The object to be weighed is placed on the left-hand pan and the weights on the right-hand pan. Weights should never be handled with the fingers but always with the forceps provided with each set of weights. A weight heavier than the object being weighed should be selected and placed on the right-hand pan, and the balance should be released. The pan with the weight should fall, showing that the weight is too heavy. The beam and pans should then be supported by turning the milled screw, the weight should be removed and replaced in the box, and the next lighter weight tried. This should prove to be too light and will therefore remain on the pan. Then the next lighter weight should be applied; if it is too heavy it should be removed, or if too light allowed to remain. By continuing this process a point is finally reached where the smallest weights have been added, and still the weights are not enough exactly to balance the object being weighed.

For the final adjustment of the balance, a small weight called a **rider,** which can be moved back and forth on the beam by a mechanical device, is utilized. This rider is placed on the beam at a point, determined by trial, where an accurate balance is obtained. The beam is calibrated, and the number where the rider rests represents milligrams that should have been added to the pan to produce a balance. Thus in adding up the weights allowance must be made for the rider, the number of milligrams indicated by the position of the rider being added to the weights on the pan. Since different balances require riders of different weights, it is important not to transfer a rider from one balance to another. A rider, when placed on the beam above the knife-edge of the pan, should have a weight in milligrams equal to the number on the beam at that point. If it becomes necessary to obtain a new rider for a balance, one that weighs a number of milligrams equal to the number on the beam above the knife-edge of the pan should be selected. Riders commonly weigh 5, 6, 8, 10, or 12 mg. After the balance has been adjusted, the final reading being taken with the window down, the weights should be accurately recorded. After making sure that the beam and pans are properly supported, the object being weighed and the weights should be carefully removed from the pans, and the weights should be placed in their proper places in the weight box.

In order to facilitate use of the rider it is desirable to know the **sensitivity of a balance,** that is, the change in zero point caused by 1 mg. of weight. It is found by determining the zero point of the balance without a load and then placing the rider at 1 on the beam and determining the zero point again. The second zero point will be to the left of the zero point without a load, the number of divisions on the scale to the left being the sensitivity of the balance. Balances differ in their sensitivity, and the sensitivity varies also with the load. It should be noted that the rider should be manipulated only while the balance is at rest and the beam and pans supported.

In the foregoing discussion the term zero point of a balance has been used This point is determined by allowing the balance to swing and noting the distance of the swing at each end of the excursion of the pointer. Four readings are taken on the left and three on the right. The average reading on each side is then calculated and the smaller number subtracted from the larger. This difference is divided by 2, the result being the zero point to the side of the center of the scale in the direction of the longer swing. The following is an example:

	LEFT		RIGHT
	2.4		2.8
	2.2		2.6
	2.0		2.4
	1.8		3) 7.8
	4) 8.4	Average	2.6
Average	2.1		2.1
			2) 0.5
		Zero point	0.25 to right

It should be noted that in the method of adjusting the balance just described, where the pointer is made to swing equal distances on each side of the center of the scale, the zero point of the balance would not be exactly at the center of the scale. Therefore in determining the sensitivity of a balance the true zero point must be determined without a load and later with a load of 1 mg.

The difference between these zero points is the sensitivity of the balance.

The capacity of most analytical balances is 200 grams. It is very unusual, however, to weigh objects heavier than 100 grams. A set of analytical weights is sufficient for weighing up to 100 grams, and if they are used properly it is not necessary to borrow weights to weigh objects of 100 grams or less.

Gravimetric Analysis. In quantitative analysis several types of procedure are employed. In many quantitative procedures the substance to be determined is converted into an insoluble compound, filtered, dried, and weighed. If the weight relationships which exist between the substance weighed and the substance being determined are known, it is possible to multiply the weight of the substance weighed by a **chemical factor,** obtaining a product which is the weight of the substance being determined. For example, if the amount of chlorine in a solution containing chloride is to be determined, the chlorine may be precipitated as silver chloride by adding an excess of silver nitrate in the presence of nitric acid. The reaction taking place may be represented as follows:

$$NaCl + AgNO_3 \rightarrow AgCl + NaNO_3$$

The silver chloride, being insoluble, may be separated by filtration and weighed. Since the molecular weight of AgCl is 143.337 and the atomic weight of Cl is 35.457, the chemical factor for converting AgCl into Cl is 35.457 ÷ 143.337 or 0.2473. Thus if 0.5000 gram of AgCl is obtained from a solution containing chloride, the amount of chlorine present in the solution is 0.5000×0.2473 or 0.12365 gram. This type of quantitative analysis is known as **gravimetric analysis.**

Volumetric Analysis. A second type of quantitative procedure, widely used in biochemical work, is known as **volumetric analysis.** In this type of analysis, solutions of known concentration, called **standard solutions,** are employed. In volumetric analysis one adds from a burette, which is an apparatus for measuring volumes of solution accurately, a standard solution of a compound which reacts with the substance to be determined. The standard solution is added until the reaction is complete, as indicated by the change in color of an **indicator.** This process of adding a standard solution is known as **titration.** If one wishes to determine chlorine volumetrically, he may do so by titrating a solution of the sample containing chloride, which has been acidified with nitric acid, with a standard solution of silver nitrate, using potassium chromate as an indicator. As silver nitrate is added, white silver chloride is precipated. When all the chloride has been precipitated, the next drop of silver nitrate reacts with the potassium chromate to form reddish brown silver chromate. This point can be readily detected by the eye and is said to be the **end point** of the titration. By noting the volume of silver nitrate used in the titration one can readily calculate the amount of chlorine in the unknown sample. For example, let us suppose that a standard solution of silver nitrate contains 0.0100 gram of $AgNO_3$ per cubic centimeter. Since one molecule of $AgNO_3$ is required to precipitate one Cl, it is evident that 0.0100 gram of $AgNO_3$ is equivalent to

$$\frac{\text{Atomic weight of Cl} \times 0.0100}{\text{Molecular weight of AgNO}_3}, \text{ or } \frac{35.457 \times 0.0100}{169.888}$$

or 0.0021 gram of Cl. Thus if 50 cc. of standard $AgNO_3$ was used in a titration, there must have been 50 × 0.0021 or 0.1050 gram of Cl in the sample.

Acidimetry and Alkalimetry. Volumetric analysis is very commonly used in titrating acids with bases, or vice versa; this kind of analysis is known as **acidimetry** or **alkalimetry.** Organic dyes which have the property of changing color near the point of neutralization serve as indicators. The concentration of standard solutions of acids and bases is expressed in terms of normality rather than in terms of the amounts of acid or base present. From the standpoint of acid and base titrations, it is the acid hydrogen of an acid or the hydroxyl group of a base that is important. The following is a typical neutralization reaction:

$$HCl + NaOH \rightarrow NaCl + H_2O$$

The process of neutralization is in reality the union of the H ion of an acid with the OH ion of a base, forming water. Thus equal volumes of solutions of acids and bases containing equal numbers of acid hydrogens and hydroxyl radicals, respectively, are equivalent or are of the same strength from the standpoint of acid-base titrations. A solution of an acid containing 1 gram atom (1.008 grams) of acid hydrogen per liter is a **normal solution of an acid.** A solution of a base containing 1 gram radical of hydroxyl (17.008 grams) per liter is a **normal solution of a base.** One cubic centimeter of a normal solution of an acid will exactly neutralize 1 cc. of a normal solution of a base. All normal solutions of acids and bases are standard solutions, but not all standard solutions of acids and bases are normal. When solutions of acids and bases are not normal, their concentrations are expressed by normality factors. A **normality factor** is a number which expresses the concentration of a solution in terms of its normality. Thus if a solution is 0.2 *N*, it is 0.2 as concentrated as a normal solution.

In making normal solutions of acids and bases, the number of acid hydrogens per molecule of acid or the number of hydroxyl radicals per molecule of base must be considered. Thus 1 gram molecule of HCl will give 1 gram atom of hydrogen, and ½ gram molecule of H_2SO_4 will give the same amount of hydrogen. Likewise 1 gram molecule of NaOH will give 1 gram radical of hydroxyl, whereas only ½ gram molecule of $Ba(OH)_2$ will be required.

To make a normal solution of HCl, 1 gram molecule of HCl is dissolved in enough water so that the final volume is 1 liter. For this purpose a liter volumetric flask is used. The proper amount of HCl is measured into the flask and water added to the mark on the neck of the flask. The total volume of liquid in the flask is then 1 liter. After thorough mixing the solution will be normal. Since HCl is a gas, and since the laboratory supply is a solution of HCl in water, the concentration of the desk reagent must be known before a normal solution can be prepared from it. Concentrated HCl has a specific gravity of 1.19 and contains 37.23 per cent of HCl. This means that 1 cc. weighs 1.19 grams and contains 37.23 per cent HCl. Therefore 1 cc. contains 1.19×0.3723, or 0.443 gram of HCl. Since it is easier to measure HCl solution than it is to weigh it, the volume of HCl which contains 1 gram molecule is calculated and this amount is measured by means of a graduate into a liter volumetric flask. Since 1 gram molecule of HCl is equal to 36.465 grams, $36.465 \div 0.443$ or 82.3 cc. of concentrated HCl will be required to make 1 liter of normal solution. For 1 liter of a 0.1 *N* solution 8.23 cc. will be required.

In a similar manner, a normal solution of NaOH is prepared by dissolving 1 gram molecule or 40.005 grams of NaOH in water and making up to a volume of 1 liter. If the NaOH is 90 per cent pure, 40.005 ÷ 0.9 or 44.45 grams will be required for a liter of normal solution, or 4.445 grams for a liter of 0.1 *N* solution. Sodium hydroxide should be weighed on a rough balance, care being taken not to allow it to come in contact with the balance or the hands.

Since it is impracticable to measure the HCl or weigh the NaOH accurately, these solutions must be standardized against some chemical of exceptional purity. Such a substance is known as a **primary standard.** Sodium carbonate serves very well as a primary standard in the standardization of an acid. To standardize a 0.1 *N* solution of HCl, a standard solution of Na_2CO_3 of approximately that strength is prepared. Sodium carbonate reacts with HCl as follows:

$$Na_2CO_3 + 2HCl \rightarrow 2NaCl + CO_2 + H_2O$$

It is thus evident that 1 gram molecule of Na_2CO_3 is equivalent to 2 gram molecules of HCl. Thus ½ gram molecule of Na_2CO_3 (53.002 grams) per liter will give a normal solution of this compound, or 5.3002 grams per liter will give a 0.1 *N* solution. For the preparation of an approximately 0.1 *N* solution of Na_2CO_3, approximately 5.3002 grams of Na_2CO_3 is weighed out on an analytical balance and dissolved in water, and the solution is transferred quantitatively to a liter flask and made up to volume.

Let us suppose that 4.8263 grams of Na_2CO_3 was weighed out. To find the exact normality of this solution, the weight of Na_2CO_3 in a liter is divided by the weight there should be in a liter of normal solution. The result is the normality factor of the solution:

$$\frac{4.8263}{53.002} = 0.0911, \text{ the normality factor of the } Na_2CO_3 \text{ solution}$$

To standardize the HCl solution one burette is filled with the standard Na_2CO_3 solution and another with the HCl solution. Approximately 25 cc. of the Na_2CO_3 solution is run into a beaker, and a drop of methyl orange indicator is added. This indicator is used because Na_2CO_3 is a weak base. (In titrating weak bases against strong acids, indicators which change color on the acid side of neutrality are used. Methyl orange changes color at a pH of 2.9 to 4.0.) The HCl solution is then added until the color changes from yellow to red. If too much HCl is added, the yellow color may be brought back with a few drops of Na_2CO_3 solution, and the end point redetermined. The volumes of Na_2CO_3 and HCl solutions used are then noted, and the normality factor of the HCl solution is calculated from these titration figures. Let us suppose that 26 cc. of Na_2CO_3 and 24 cc. of the HCl solution are used. The calculations will be as follows:

$$\begin{array}{ccccccc} \text{cc. } Na_2CO_3 & : & \text{cc. HCl} & :: & N \text{ factor of HCl} & : & N \text{ factor of } Na_2CO_3 \\ 26 & : & 24 & :: & x & : & 0.0911 \end{array}$$

$$x = 0.0987, \text{ the } N \text{ factor of the HCl}$$

The HCl solution is now accurately standardized and may be used for standardizing the NaOH solution. This is done by replacing the standard solution of Na_2CO_3 in the one burette with the solution of NaOH to be standardized. The foregoing process is then repeated, and the titration figures are used in calculating the normality of the NaOH solution. Let us suppose that 30 cc. of the NaOH solution is required to neutralize 27 cc. of the HCl solution. The calculations will then be as follows:

$$\begin{array}{ccccccc} \text{cc. NaOH} & : & \text{cc. HCl} & :: & N \text{ factor of HCl} & : & N \text{ factor of NaOH} \\ 30 & : & 27 & :: & 0.0987 & : & x \end{array}$$

$$x = 0.0888, \text{ the } N \text{ factor of the NaOH}$$

Standard solutions of acid and base are used largely for determining the amount of base or acid in unknown solutions. Let us suppose that we wish to determine the percentage of acetic acid in vinegar by means of titration with a standard solution of base. For this determination we would measure accurately from a pipette 5 cc. of vinegar into a beaker, add a drop of indicator, in this case phenolphthalein, because we are titrating a weak acid against a strong base, and titrate to the appearance of a pink color with standard NaOH solution. In titrating weak acids, such as acetic acid, with strong bases, indicators which change color on the alkaline side of neutrality are used. Phenolphthalein changes color at a pH of 8.3 to 10.0. Let us suppose that 40 cc. of NaOH (0.0888 N) is required to neutralize the acid in this sample and that the specific gravity of the sample is 1.052.

Since the specific gravity of the vinegar is 1.052, the 5-cc. sample will weigh 1.052 × 5 or 5.260 grams. In this sample there is enough acetic acid to neutralize 40 cc. of 0.0888 N NaOH solution. This is equivalent to 40 × 0.0888 or 3.5520 cc. of normal NaOH. Since 1 cc. of normal NaOH is equivalent to 1 cc. of normal acetic acid, it is evident that in the sample of vinegar there is acetic acid equivalent to 3.5520 cc. of normal acetic acid. Since CH_3COOH has 1 acid hydrogen per molecule, 1 gram molecule (60.052 grams) will be present per liter of normal solution. One cubic centimeter of normal acetic acid solution will then contain 0.06001 gram of acetic acid. Thus the sample of vinegar will contain 3.5520 × 0.06001 or 0.2135 gram of acetic acid. To determine the percentage of acetic acid in the sample, this figure is multiplied by 100 and divided by the weight of the sample, thus:

$$\frac{0.2135 \times 100}{5.260} = 4.06 \text{ per cent acetic acid}$$

These calculations may be simplified by the use of the following formula:

$$\frac{\text{cc. NaOH} \times N\text{.F. of NaOH} \times \dfrac{\text{Weight of } CH_3COOH}{\text{in 1 cc. } N} \times 100}{\text{cc. of sample} \times \text{Specific gravity}} = \%\text{ acetic acid}$$

or

$$\frac{40 \times 0.0888 \times 0.0601 \times 100}{5 \times 1.052} = 4.06 \text{ per cent acetic acid}$$

Determination of the amount of acetic acid in vinegar is important in connection with food laws. The minimum percentage of acetic acid in vinegar is regulated by federal and state food laws. The Federal Food and Drug Administration states that cider or apple vinegar should contain in 100 cc. at 20°C. not less than 4 grams of acetic acid.

In a similar manner the percentage of sodium carbonate in a washing soda may be determined. A sample of the washing soda is weighed out accurately on an analytical balance, dissolved in water, and titrated with standard acid, using methyl orange as an indicator. Let us suppose that a 0.3000-gram sample of washing soda requires 50 cc. of 0.0987 N HCl for neutralization. The amount of normal acid required for neutralization will then be 50 × 0.0987 or 4.9350 cc. Since 1 cc. of normal acid is equivalent to 1 cc. of normal Na_2CO_3 solution, there must have been enough Na_2CO_3 in the sample to make 4.9350 cc. of normal solution. Since 1 liter of normal Na_2CO_3 solution contains 53.002 grams of Na_2CO_3, 1 cc. will contain 0.0530 gram, and 4.9350 cc. will contain 4.9350 × 0.0530 or 0.2616 gram. This figure multiplied by 100 and divided by the weight of the sample gives the percentage of Na_2CO_3 in the sample, thus:

$$\frac{0.2616 \times 100}{0.3000} = 87.2 \text{ per cent } Na_2CO_3$$

These calculations may be simplified by the use of the following formula:

$$\frac{\text{cc. HCl} \times N\text{.F. of HCl} \times \text{Weight of } Na_2CO_3 \text{ in 1 cc. } N \times 100}{\text{Weight of sample}} = \%\ Na_2CO_3$$

or

$$\frac{50 \times 0.0987 \times 0.0530 \times 100}{0.3000} = 87.2 \text{ per cent } Na_2CO_3$$

Colorimetry. A third type of analytical procedure used extensively in biochemical work is known as **colorimetry.** In colorimetry the sample is treated with a reagent which produces a color with the constituent to be determined. A standard solution of the substance to be determined is treated in the same way, and the colors of the standard and of the unknown are compared in an instrument called a **colorimeter.** The intensity of the color is directly proportional to the concentration of the colored substance. According to **Beer's law,** light in passing through a colored solution is absorbed in direct proportion to the concentration of the colored substance in solution and in inverse proportion to the depth of the observed layer. Thus a 20-mm. layer of a solution of a colored compound will give the same intensity of color as a 10-mm. layer of a solution of the same compounds of twice the concentration.

A colorimeter (see Fig. 2) is an instrument with cups fitted onto a stage which may be moved up and down. Above each cup

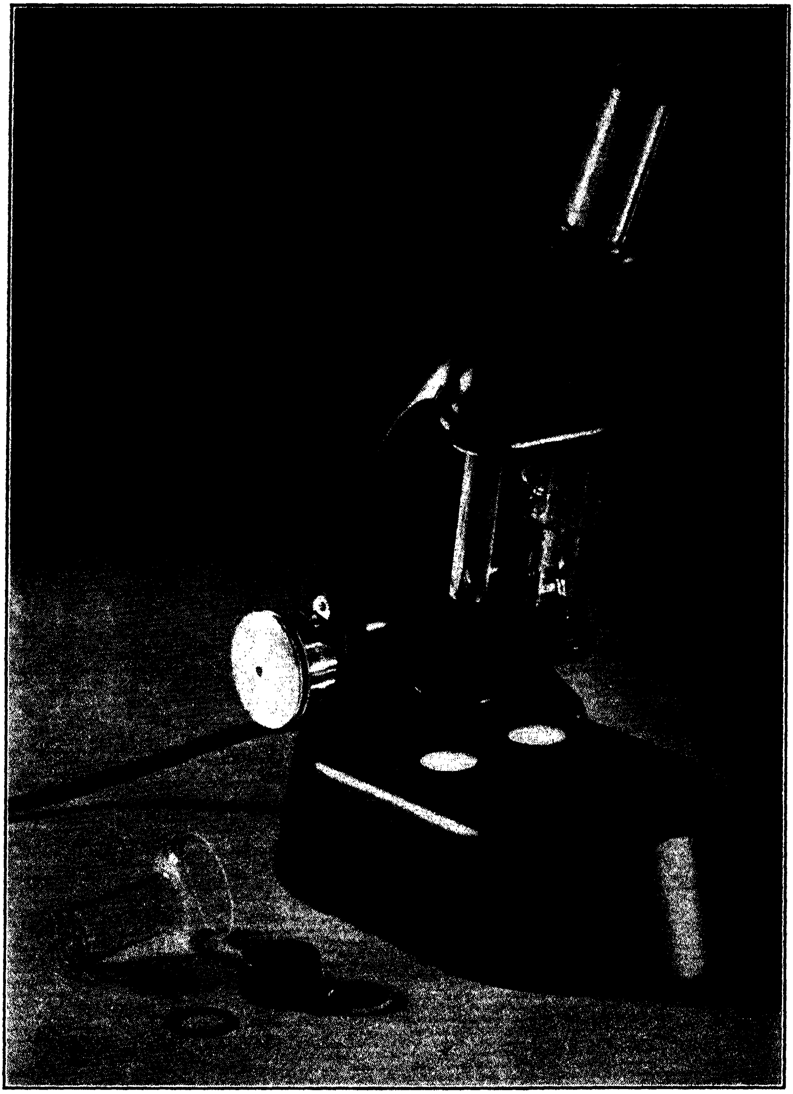

FIG. 2. Colorimeter. Courtesy of the Spencer Lens Company.

is a glass rod or plunger which fits into each cup as the cup is elevated. By means of an optical system it is possible to look through the plungers. When a cup is raised so that the bottom of it is in contact with the bottom of the plunger, the scale on the mechanical device for raising the cup reads zero. In using a colorimeter a cup is half filled with the solution under examination, and the plunger is dipped into the solution. If the cup is filled, the solution will overflow when the plunger is introduced. If the cup is raised until the scale reading is 20 mm., there is a layer of solution 20 mm. deep under the plunger. In actual practice the standard solution is placed in the left-hand cup and the scale set at 20 mm. The unknown solution is then placed in the right-hand cup, which is moved up and down until the color of the unknown matches the color of the standard, as indicated by a field of uniform intensity of color in the eyepiece. The depth in millimeters of the unknown solution is then determined by reading the scale, and the data obtained are used in calculating results by means of the following formula:

$$\frac{\text{Reading of standard} \times \begin{matrix}\text{Weight of unknown}\\ \text{in standard}\end{matrix}}{\text{Reading of unknown}} = \text{Weight of unknown in sample}$$

Thus in a sugar determination if we used 2 cc. of a standard containing 0.2 mg. of glucose and 2 cc. of an unknown and the colorimeter readings were 20 mm. for the standard and 15 mm. for the unknown, the calculations would be as follows:

$$\frac{20 \times 0.2}{15} = 0.267 \text{ mg. of glucose in 2 cc. of the unknown}$$

Colorimetry is of especial value in determining substances which are present in only small amounts. Since traces of highly colored compounds give highly colored solutions, methods of analysis based on color comparisons are of great value in determining many compounds present in low concentrations. Creatinine occurs in blood to the extent of 1 to 2 mg. per 100 cc., yet its concentration may be accurately determined on a 1-cc. sample of blood.

A good colorimeter is an expensive piece of apparatus and should be treated very carefully. Cups should never be filled to the extent that there is danger of overflowing. If overflowing does occur, the instrument should be thoroughly cleaned immediately. Many of the solutions compared in a colorimeter are corrosive and will destroy the mirror which reflects the light into the base of the cups or will corrode the cup supports. After a colorimeter is used, the cups should be rinsed thoroughly with distilled water. The plungers should also be rinsed by dipping them in several changes of distilled water in the cleaned cups. Finally the plungers should be carefully dried with a clean cloth.

For accurate results in colorimetry it is important that the instrument be in proper adjustment. An accurately adjusted colorimeter should give identical readings with the two cups when both contain the same solution. If the colors do not match when both cups are set at the same scale readings, adjustments must be made before proceeding.

Another test which should be applied to a colorimeter before using is to make sure that both cups read zero when they are elevated so that their bottoms are in contact with the plungers. Also the mirror and the light should be adjusted so that the two halves of the field observed through the eyepiece appear uniform and identical.

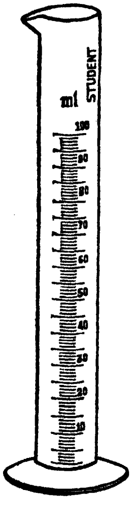

Fig. 3. Graduate.

Volumetric Apparatus. Since in quantitative analysis frequent use is made of volumetric apparatus, the worker should be familiar with the types of such apparatus commonly employed.

Graduated cylinders or **graduates** (see Fig. 3) are used only for measuring liquids roughly. They should never be employed where exact amounts are involved.

Pipettes are used to measure exact amounts of liquids. As a rule they are calibrated to deliver a definite volume of liquid. Sometimes they are calibrated to contain a definite volume; if so, they must be rinsed out when used.

Pipettes are of two types (see Fig. 4), graduated and bulb. Graduated pipettes are of uniform bore and have graduations along the side. They are used for measuring small amounts of liquid, and the amount delivered is indicated by reading the calibrations on the side of the pipette.

The more common type of pipette is a bulb pipette, which has a mark above the bulb. When filled to this mark, the pipette will deliver the volume indicated on the bulb. In using a pipette the tip is placed in the liquid to be measured, and the liquid is drawn into the pipette by suction from the mouth. Extreme caution should be taken not to draw liquid into the mouth. This may be avoided by making sure that the tip of the pipette is well below the surface of the liquid. The liquid should be drawn above the mark and the index finger quickly placed over the top of the pipette. As the finger is carefully released, the liquid will run down to the mark. Any drop of liquid on the end of the pipette should then be removed by touching it to the side of the vessel containing the liquid. The pipette should be allowed to drain by holding it in a vertical position; draining should not be hastened by blowing through the pipette. A good method of

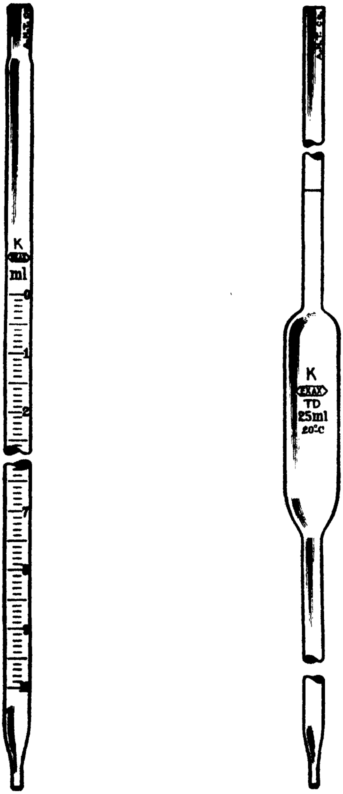

FIG. 4. Graduated and bulb pipettes.

obtaining the last drop from the pipette is to place the index finger of the right hand over the top end, grasping the bulb with the left hand. The heat of the hand will expand the air in the bulb, forcing the last drop out.

Graduated flasks are made to contain a definite volume of liquid when filled to a mark on the neck of the flask. (See Fig. 5.) They are used for making standard solutions or whenever an aliquot part of a sample is desired. Thus, if a sample is dissolved in water and the volume is made up to 500 cc., a 100-cc. aliquot of this solution will contain one-fifth of the original sample.

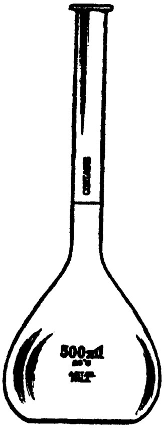

Fig. 5. Graduated flask.

Burettes are long tubes which are graduated in tenths of a cubic centimeter. (See Fig. 6.) They are used mainly in titrations in which the volume of liquid to be added is not known but in which one must know exactly how much liquid has been added when the titration is complete. It should always be ascertained that there are no air bubbles in the tip of a burette before titration is begun.

A burette may be read accurately to 0.02 cc. It will be noted in reading that the surface of the liquid is not flat, but curved. This curved surface is called the **meniscus,** and it is customary to read the bottom of the meniscus. Reading is facilitated by holding a piece of paper against and behind the burette. The eye should be on a level with the bottom of the meniscus.

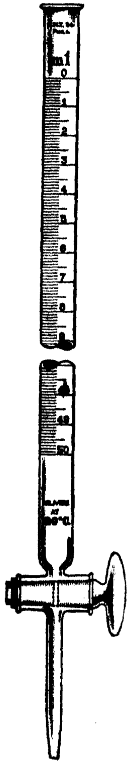

Fig. 6. Burette.

Cleaning Apparatus. It is very important that apparatus used for measuring liquids accurately be clean. Soap and water and brushes are best to use for cleaning glassware, except pipettes, for which cleaning fluid is employed. This fluid is prepared by dissolving 25 grams of sodium or potassium dichromate in 100 cc. of water and adding 100 cc. of concentrated sulfuric acid. As this solution can be used repeatedly, it should not be discarded after using once. It should be kept in a glass-stoppered bottle. To clean pipettes, they should be filled with cleaning fluid several times. Extreme care should be taken not to get the fluid into the mouth. A clean pipette, like any other kind of glassware, will drain without drops of liquid adhering to its side. A common method of cleaning pipettes is to allow them to stand in a tall cylinder of cleaning fluid over night.

Laboratory Records. The experiments which follow are designed to aid the student in understanding the work covered in the textbook. A notebook in which accurate details of the results obtained in the laboratory are recorded should be kept. Accompanying each experiment are questions whose purpose is to bring out the more important points which the student should learn from performing the experiment. The answers to these questions should be an important part of the laboratory record. Since many of the questions may be answered by observing closely what happens while performing the experiment, it is well to read the questions first. Many of them may be answered by careful study of the text.

General conduct in the laboratory is an important factor in determining a student's success in laboratory work. Neatness, careful technique, accuracy, and thoroughness are vital. It is better to do a few experiments well, to have a neat desk and clean apparatus, and to understand thoroughly what has been done than to perform many experiments in a slipshod manner.

PROBLEMS

1. How many cubic centimeters of HCl having a specific gravity of 1.19 and containing 37.23 per cent HCl by weight will be required to make 2500 cc. of 0.2350 *N* solution?

2. Two grams of Na_2CO_3 is dissolved in water and made up to a volume of 500 cc. Calculate the normality of the Na_2CO_3 solution.

3. What weights of the following acids must be dissolved in 1500 cc. to make a 0.2 *N* solution: H_2SO_4, H_3PO_4, HNO_3, and CH_3COOH?

4. What weights of the following bases must be dissolved in 250 cc. to make a 0.05 *N* solution: KOH, $Ca(OH)_2$, and $Ba(OH)_2$?

5. A Na_2CO_3 solution has a normality of 0.2150. Twenty cubic centimeters of this solution is required to neutralize 25 cc. of HCl solution. What is the normality of the HCl solution?

6. One gram of washing soda requires 30 cc. of 0.1 *N* HCl for neutralization. Calculate the percentage of Na_2CO_3 in the washing soda.

7. Five cubic centimeters of vinegar, specific gravity 1.052, requires 12 cc. of 0.18 *N* NaOH for neutralization. Calculate the percentage of acetic acid in the sample.

QUANTITATIVE ANALYSIS

Before proceeding with the experiments in this section, read the preceding chapter carefully.

Experiment 1*

Determination of the Sensitivity of a Balance

Very cautiously turn the milled screw in the center of the base of the balance to the left, bringing the knife-edges of the beam in contact with their bearing surfaces. Next push in the button to the left of the milled screw, which removes the pan supports. The beam should now swing, the pointer passing over several divisions of the scale at each excursion. After a few excursions of the pointer begin to note and record the distance, estimated in tenths of scale divisions, which the pointer swings to either side of the center of the scale. Take four readings to the left and three to the right, placing the values in two columns headed L and R. Add the two columns, and divide the first sum by 4 and the second by 3 to obtain the average. Subtract the smaller average from the larger and divide this result by 2. The result is the distance from the center of the scale, expressed in scale divisions, on the side of the longer swing, at which the pointer would come to rest if allowed to do so. This is the zero point of the balance without a load. The zero point should be close to the center of the scale; if it is more than one scale division off, adjust the balance by turning the nut on the end of the beam. If this adjustment is found necessary, redetermine the zero point after the adjustment has been made.

Next bring the beam to rest by turning the milled screw to its original position as the pointer passes the center of the scale. Support the pans by allowing the button to the left to come out. Now place the rider on the right arm of the beam at the point marked 1. This is equivalent to adding a milligram weight to the right pan. Determine the zero point as above. It will be found to be to the left of the previous zero point. The difference between the two zero points, expressed in scale divisions, is the sensitivity of the balance.

QUESTIONS

1. Define the zero point of a balance.
2. Define the sensitivity of a balance.
3. Does the sensitivity of a balance vary with the load on the pans?
4. How would you determine the weight of the rider to use on a balance?
5. Give data obtained and show calculations for the determination of the zero point and the sensitivity of your balance.

Experiment 2*

Practice in Weighing

Adjust the balance so that the pointer swings equal distances to each side of the center division on the scale. By means of a forceps place a coin on the center of the left-hand pan of the balance. Next, using forceps, remove a weight from your set which you estimate to be just too heavy to balance the coin. Place this on the center of the right-hand pan and release the balance as described in Experiment 1. If the weight is too heavy, as is to be expected, remove it after the balance is brought to rest, and test the next lighter weight in the same way. If this is not heavy enough, leave it on the pan and add the next lighter weight, removing it if too heavy and replacing it by the next lighter weight. Continue this process until it is necessary to use the rider. At this point close the balance window, and adjust the rider until the pointer again swings equal distances on both sides of the center of the scale. It should be emphasized that no weights should be added or removed from the pan or the rider adjusted when the balance is not at rest. Record the weights, including the rider; remove the rider, and replace the weights in their proper places in the weight box. Next weigh another coin in exactly the same manner, and record its weight. Finally weigh the same two coins together. The sum of the weights of the two coins should equal the weight of the coins together. These weights should of course check exactly, but for our purposes an error of 0.0005 gram is permissible. If your results do not check, it indicates that you have made an error in weighing or that your weights are not accurate.

Do not borrow weights from your neighbor's balance. If your set of weights is complete, you have sufficient weights to weigh any object up to 100 grams. If your set of weights is incomplete or inaccurate, see the instructor.

QUESTIONS

1. What is the total weight of the weights in your set?
2. Why is it important not to handle weights with the fingers?
3. What is the weight of each coin, and what is the sum of the weights of both coins?
4. What is the weight of the two coins weighed together?
5. What is the difference between the sum of the weights of the two coins and the weight of the two coins weighed together?

Experiment 3 *

Preparation of a Standard Solution of Sodium Carbonate

Obtain from the instructor a few grams of pure anhydrous Na_2CO_3 which has been dried for several hours in an oven at 100°C. This sample should be placed in a stoppered weighing bottle, and if not already cool it should be cooled before weighing.

Calculate the weight of Na_2CO_3 necessary to make 500 cc. of 0.1 *N* solution, keeping in mind that ½ gram molecule per liter gives a normal solution. (See page 8.) Weigh the weighing bottle with its Na_2CO_3 accurately, and record the weight. By means of a spatula, transfer to a clean beaker, a quantity of Na_2CO_3 which you think will weigh the required amount. Weigh the weighing bottle again. The loss in weight is equal to the weight of the sample removed. This should be within ½ gram of the desired amount. Next dissolve the Na_2CO_3 in the beaker in a small amount of distilled water. Place a funnel in a 500-cc. graduated flask, and pour the Na_2CO_3 solution into the funnel. By means of a wash bottle rinse the last trace of the Na_2CO_3 solution into the funnel with several rinsings. Finally rinse the funnel inside and its stem outside, collecting all the rinsings in the flask. Then add water to the mark, stopper, and shake thoroughly. The solution is a standard solution of Na_2CO_3, which will be used in Experiment 4. To calculate the normality of this solution, divide the weight of Na_2CO_3 used by the amount of Na_2CO_3 necessary for 500 cc. of normal solution. The result is the normality factor of the Na_2CO_3 solution. Normality factors should be calculated to the fourth decimal place. Since this solution is the starting point for several subsequent experiments, great care must be taken in its preparation. An error here will of course be reflected in all work in which this solution is employed as the primary standard.

QUESTIONS

1. Write an equation representing what happens when Na_2CO_3 reacts with HCl.
2. Why must the dry Na_2CO_3 be kept in a stoppered weighing bottle?
3. Why must the Na_2CO_3 be cool before weighing?
4. What weight of Na_2CO_3 is necessary for 500 cc. of normal solution?
5. What weight is necessary for 500 cc. of 0.1 *N* solution?
6. Give your data for calculating the normality of your Na_2CO_3 solution.

Experiment 4 *

Preparation and Standardization of an Approximately 0.1 *N* Solution of Hydrochloric Acid

Preparation. Calculate the volume of concentrated HCl necessary for 1 liter of 0.1 *N* solution. (See page 7.) Concentrated HCl has a specific gravity of 1.19 and contains 37.23 per cent HCl by weight. Measure in a graduate the calculated amount of HCl, and transfer it to a bottle of 1-liter capacity. By means of a large graduate add to the HCl in the bottle sufficient distilled water to make a total volume of 1 liter. Shake thoroughly. This should be an approximately 0.1 *N* solution of HCl.

Standardization. Thoroughly clean 2 burettes by means of soap and water and a burette brush. Rinse thoroughly with water. If the burettes are clean, water will not adhere to the inside in drops when they are drained but will form an even film over the surface. Fill the first burette with standard Na_2CO_3 solution and the second with HCl solution to be standardized. If the burettes are wet, they should be rinsed with the solution with which they are to be filled in order to prevent dilution. Fill the burettes to above the graduations, and by opening the stopcock allow the liquid to drain into a beaker until the bottom of the meniscus is at the first graduation mark of each burette. At this point there should be no air bubbles in the tips of the burettes.

Transfer to a clean Erlenmeyer flask about 25 cc. of distilled water and 1 drop of methyl orange indicator. From the burette add approximately 20 cc. of the HCl solution. Next place the flask under the burette containing the standard Na_2CO_3 solution. It is advisable to place a piece of white paper under the flask to aid in observing the end point of the titration. Add rapidly about 15 cc. of the Na_2CO_3 solution and then continue adding

it slowly, with constant agitation of the flask, until a final drop will dissipate the red color, leaving the solution a golden brown. This is the end point of the titration. If too much Na_2CO_3 solution is added, as indicated by a lemon yellow color, add a few drops of the HCl solution from the burette, and more cautiously redetermine the end point by adding more Na_2CO_3 solution from the burette. When the end point has been reached, carefully read the burettes, estimating to 0.01 cc., and record the results. Calculate the normality factor of the HCl solution by means of the following formula:

$$\text{cc. } Na_2CO_3 \text{ used} : \text{cc. HCl used} :: x : N.\ F. \text{ of } Na_2CO_3$$
$$x = \text{normality factor of the HCl solution}$$

Repeat the titration until values for the normality factor of the HCl solution check to the third decimal place. The average of several titrations which check closely should be used as the normality factor of the solution. Place a label on the bottle of standard HCl solution. Write on the label "Standard HCl Solution," the normality factor, date of standardization, indicator used, and the information that it was standardized against Na_2CO_3. When the data are available after the next experiment is completed, state on the label the value of 1 cc. of HCl solution in terms of your standard NaOH solution and also the value of 1 cc. of the NaOH solution in terms of the standard HCl solution. If the standard HCl solution is kept in a well-stoppered bottle, it should not change in strength for a considerable period of time.

QUESTIONS

1. Define normal solution of an acid.

2. Why is it not advisable to use phenolphthalein in standardizing a strong acid against Na_2CO_3?

3. Over what range of pH does methyl orange change color?

4. What is the difference between a normal solution and a standard solution?

5. How many grams of the following acids would be required for 250 cc. of a normal solution: CH_3COOH, H_2SO_4, H_3PO_4?

6. What is meant by a normality factor?

7. What is an indicator?

8. How could you standardize a solution of HCl gravimetrically?

9. Why must air bubbles be removed from the tip of a burette before using?

10. Give your data and show how you calculated the normality factor of your HCl solution.

11. One cubic centimeter of your HCl solution contains what weight of HCl?

Experiment 5 *

Preparation and Standardization of an Approximately 0.1 *N* Solution of Sodium Hydroxide

Preparation. Considering the laboratory supply of NaOH to be 90 per cent pure, calculate the weight necessary to make 1 liter of 0.1 *N* solution. (See page 8.) NaOH, being very corrosive, should be handled with crucible tongs and should not be allowed to come in contact with the hands, clothes, or balance. Place a small beaker on a rough balance (not an analytical balance), and balance with weights. Add enough weights to equal the weight of NaOH required. Then add the NaOH to the beaker until the weights are balanced roughly. Dissolve the NaOH in 100 cc. of distilled water. Transfer this solution to a 1-liter bottle and add 900 cc. of distilled water, using part of the water to rinse out the beaker, adding the rinsings to the bottle. Stopper the bottle with a rubber stopper, and shake thoroughly. The solution is now an approximately 0.1 *N* solution of NaOH.

Standardization. Fill one burette with the standard HCl solution and the other with the NaOH solution to be standardized Place about 25 cc. of distilled water and a drop of methyl orange in an Erlenmeyer flask, and add, from the burette, about 20 cc. of standard HCl solution. Next add NaOH solution from the other burette until the same end point is reached as described in Experiment 4. Carefully read each burette, and from the data obtained calculate the normality factor of the NaOH solution by use of the following formula:

$$\text{cc. of HCl used} : \text{cc. NaOH used} :: x : N.\ \text{F. of HCl}$$
$$x = \text{normality factor of the NaOH}$$

Repeat the titration until results check to the third decimal place. Put a label on the bottle of NaOH solution with the corresponding information required for the standard HCl solution. To find the value of 1 cc. of NaOH solution in terms of HCl solution, divide the number of cubic centimeters of HCl solution used in the foregoing titration by the number of cubic centimeters of NaOH

solution used. To find the value of 1 cc. of HCl solution in terms of NaOH solution, divide the number of cubic centimeters of NaOH solution used in the foregoing titration by the number of cubic centimeters of HCl solution used.

QUESTIONS

1. Calculate the weights of pure KOH and $Ba(OH)_2$ necessary for 500 cc. of 0.5 *N* solution of each.

2. Why is it not advisable to keep alkali in a glass-stoppered bottle?

3. Give your data, and show how you calculated the normality factor of your NaOH solution.

4. One cubic centimeter of your NaOH solution contains what weight of NaOH?

5. One cubic centimeter of your NaOH solution is equivalent to how many cubic centimeters of the HCl solution?

6. One cubic centimeter of your HCl solution is equivalent to how many cubic centimeters of your NaOH solution?

7. What use may be made of the data in 5 and 6?

8. Forty cubic centimeters of 0.5 *N* HCl solution are equivalent to how many cubic centimeters of 0.1 *N* NaOH solution?

Experiment 6 *

Determination of the Total Acidity of Vinegar

From the instructor obtain in a dry flask the sample of vinegar to be analyzed. By means of a dry 5-cc. bulb pipette transfer 5 cc. of the vinegar to an Erlenmeyer flask. A wet pipette may be used if it is first rinsed several times with the sample which is to be measured, the rinsings being discarded. Boil some distilled water to remove CO_2. When CO_2 is dissolved in water, it forms H_2CO_3, which titrates as a weak acid. Cool the boiled water, and then add 50 cc. of it to the 5-cc. vinegar sample.

Add a few drops of phenolphthalein indicator, and titrate with your standard NaOH solution to the appearance of the first permanent pink color. Read your burette, and record the number of cubic centimeters of NaOH solution used. Repeat the titration on other 5-cc. samples of vinegar until titrations check within about 0.1 cc. Using the average value of titrations which check closely, calculate the percentage of acetic acid in the vinegar. For this calculation it will be necessary to determine the specific gravity of the vinegar by pouring some of it into a graduated cylinder and placing in it a hydrometer. The specific gravity is then read directly by noting the point on the hydrometer scale

corresponding to the surface of the liquid. For your calculations, substitute in the following formula:

$$\frac{\text{cc. NaOH} \times N\text{.F. of NaOH} \times \frac{\text{Weight of acetic}}{\text{acid in 1 cc. } N} \times 100}{\text{cc. of sample} \times \text{Specific gravity}} = \%\text{ of acetic acid}$$

QUESTIONS

1. Why is recently boiled water used in diluting the vinegar?
2. Why is phenolphthalein used as the indicator in this titration?
3. At what *p*H value does phenolphthalein turn pink?
4. What percentage of acetic acid should there be in vinegar?
5. What is the origin of the acetic acid in vinegar?
6. Using the above formula, substitute your data, and calculate the percentage of acetic acid in your sample.

Experiment 7 *

Determination of the Total Alkalinity of Washing Soda Expressed as Percentage of Sodium Carbonate

From the instructor obtain a sample of washing soda in a dry weighing bottle. Weigh the bottle with the sample, and record the weight. By means of a spatula transfer about 0.2 gram to an Erlenmeyer flask, and weigh the bottle again, recording the weight. The difference between the two weights is the weight of the sample to be analyzed. Dissolve the sample in the Erlenmeyer flask in 50 cc. of distilled water, add 1 drop of methyl orange indicator, and titrate with your standard HCl solution to a golden brown end point. From the amount of the HCl solution used, calculate the percentage of Na_2CO_3 in the sample by using the following formula:

$$\frac{\text{cc. HCl} \times N\text{.F. of HCl} \times \text{Weight of } Na_2CO_3 \text{ in 1 cc. } N \times 100}{\text{Weight of sample}} = \%\text{ of } Na_2CO_3$$

Repeat the experiment until results check within 0.2 per cent.

QUESTIONS

1. Why is methyl orange used as the indicator in this titration?
2. What is the formula for washing soda? For baking soda?
3. Why must the weighing bottle be dry before your sample is obtained?
4. What is the chemistry involved in the softening of water by means of washing soda?
5. Using the above formula, substitute your data and calculate the percentage of Na_2CO_3 in your sample.

PHYSICAL CHEMISTRY

Experiment 8

Demonstration of Osmotic Pressure[1]

Thoroughly clean a small Erlenmeyer flask and dry it. Introduce into the flask about 5 cc. of collodion and rotate the flask, allowing the collodion to spread in a thin layer over the entire inner surface, and continuing the rotating until the collodion is set. The collodion layer should be fairly thick for good results. If necessary, add more collodion and repeat the process.

Allow the flask to stand until the collodion is dry; it may stand until the next laboratory period. When dry, fill the flask with water and let it stand for a few minutes. When the collodion has absorbed enough water to become pliable, pour off the water, and gently loosen the collodion membrane around the neck of the flask. By introducing water between the membrane and the flask it is possible to loosen the entire membrane. Remove it, and fill it with water to test it for imperfections. Next fit the neck of the membrane over a two-hole rubber stopper of a size to fit the Erlenmeyer flask in which the membrane was made. (See Fig. 7.) Through one of the holes of the stopper pass a glass tube about 2 ft. long. Tie the membrane to the stopper with a string, and seal the membrane to the stopper with collodion. Through the other hole in the stopper introduce a 0.5 M[2] solution of sucrose by means of a pipette, filling the membrane and making

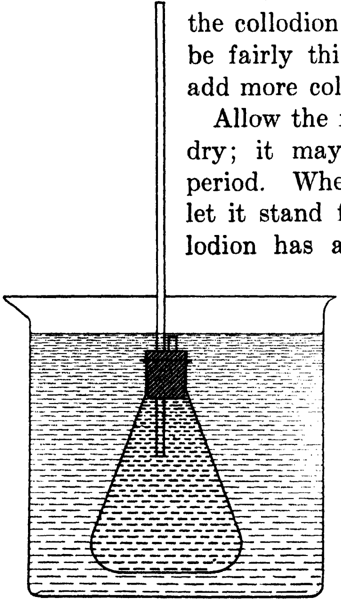

FIG. 7. Apparatus for demonstrating osmotic pressure.

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[1] Because of the difficulty in making a satisfactory membrane it is suggested that this experiment be performed by the instructor for the class.

[2] In a molal solution 1 gram molecule is dissolved in 1000 grams of solvent rather than being contained in 1000 cc. of solution, as in a molar solution.

(*Footnote continued on page 36*)

sure that there are no air bubbles in it. Stopper the hole in the rubber stopper with a short glass rod. Suspend the membrane in a beaker of distilled water so that the level of the water is even with the top of the rubber stopper. By means of a clamp attached to the glass tube, hold the membrane in place. Mark the level of the sugar solution in the glass tube. Note the level of the liquid in the tube at the end of 15 minutes and of half an hour.

QUESTIONS

1. How many inches did the liquid rise at the end of 15 minutes? Half an hour?

2. What is the osmotic pressure, in atmospheres, of a 0.5 *M* solution of sucrose?

3. If the membrane were perfectly semipermeable and dilution need not be considered, how high in meters would the liquid rise eventually, considering the liquid in the tube to be 1/13 the weight of mercury?

4. What would you expect the freezing point and the boiling point of the sucrose solution to be?

5. What do you understand by colligative properties of solutions?

Experiment 9 *

Freezing Point of Solutions

Fill a beaker with a mixture of crushed ice and salt, and introduce into it a large test tube fitted with a 2-hole rubber stopper. Through one hole insert a thermometer and through the other a glass tube bent at the bottom and small enough so that it may be moved up and down as an agitator. (See Fig. 8.) Introduce into the test tube 25 cc. of water,

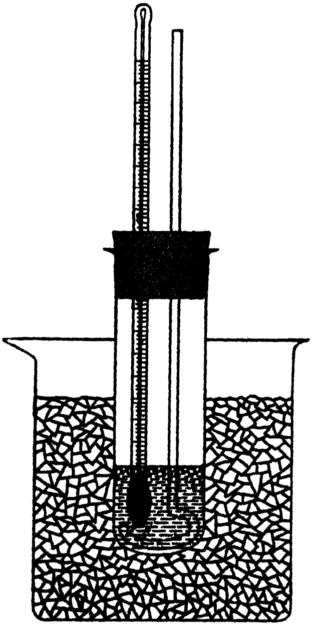

FIG. 8. Apparatus for determining the freezing point.

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* A molecule of sucrose when dissolved in water takes on 6 molecules of water, forming a hexahydrate. Therefore, to make a liter of a 0.5 *M* solution of sucrose, 171 grams of sucrose is added to 1054 cc. of water. The 54 cc. of water combines with the sucrose to form the hexahydrate. The same concentration will be obtained by adding 162.24 grams of sucrose to 1 liter of water.

and constantly agitate the water by means of an up-and-down motion of the glass rod. Carefully observe the temperature of the water as it cools. The temperature should go below 0°C., and when freezing starts there should be a sharp rise in temperature to a definite point. When all the water is frozen, the temperature will decrease again. Record the thermometer reading at which the temperature is constant shortly after freezing starts. This is the freezing point of water. Repeat the experiment with a 0.5 *M* solution of sucrose and a 0.5 *M* solution of NaCl.[3]

QUESTIONS

1. What are the freezing points observed for water, sucrose, and NaCl solutions?
2. What would you expect the freezing point of the sucrose solution to be?
3. How do you account for the fact that it is slightly lower than it should be?
4. How do you account for the low freezing point of the NaCl solution?
5. What is the freezing point of milk?
6. How would water added to milk affect its freezing point?
7. From your observed freezing point of the NaCl solution, calculate its osmotic pressure and its boiling point.

Experiment 10

Surface Tension

Support a fine needle in two loops of thread, and gently lower it onto the surface of water in a beaker so that the needle floats. Notice the depression in the surface of the water caused by the needle. By means of a pipette run a few drops of 1 per cent soap solution down the side of the beaker.

QUESTIONS

1. Define surface tension.
2. What happens to the needle when the soap is added? Explain.
3. Would you expect NaCl to lower or increase the surface tension of water?
4. What is adsorption?

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[3] A 0.5 *M* solution of NaCl is made by dissolving 29.2 grams of NaCl in 1 liter of water.

Experiment 11

Colorimetric Determination of *p*H

Obtain from the instructor a sample the *p*H of which is to be determined. In preliminary tests determine its approximate *p*H value by testing a few drops on a spot plate with various indicators. For example, if no color is obtained with phenolphthalein, the *p*H is less than 8.3. If the sample is red to litmus, the *p*H is less than 7.0. If yellow to bromothymol blue, the *p*H is less than 6.0. If yellow to alizarin red, the *p*H is less than 5.5. If red to methyl red, the *p*H is less than 4.4. If purple to alizarin red, and yellow to bromothymol blue, the *p*H must be between 5.5 and 6.0. Table 1 gives the *p*H values at which various indicators change color.

When the approximate *p*H of the solution has been determined, arrange about 6 or 8 test tubes in your test-tube rack and place in the tubes a series of buffer solutions[4] covering the color range of the indicator whose color change occurs at the *p*H of the unknown solution. For example, if your preliminary tests indicate that the *p*H of your solution is between 5.5 and 6.0, alizarin red may be selected as the indicator. Place 5 cc. of buffer solutions in the test tubes, beginning with a *p*H of 5.4 and ending with a *p*H of 6.8. Next place 5 cc. of the unknown solution in a test tube, and add to each tube exactly 1 drop of alizarin red from a pipette. The buffer solutions should range in color from yellow

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[4] Buffer solutions covering a range of *p*H from 3.0 to 10.0 may be made by mixing the following solutions and making up to a volume of 200 cc.

*p*H	cc. 0.2 *M* KHPhthalate	cc. 0.2 *M* HCl
3.0	50	20.32
3.2	50	14.70
3.4	50	9.90
3.6	50	5.97
3.8	50	2.63
		cc. 0.2 *M* NaOH
4.0	50	0.40
4.2	50	3.65
4.4	50	7.35
4.6	50	12.00
5.0	50	23.65
5.2	50	29.75
5.4	50	35.25
5.6	50	39.70

to purple, and the unknown should have a color which closely matches one of these hues. The *p*H of the buffer solution which matches color with the unknown indicates the *p*H of the unknown. Estimate the *p*H to 0.1.

QUESTIONS

1. What is the *p*H of your solution?
2. What indicator did you use, and what is its color range?
3. Define *p*H.
4. How may *p*H be determined electrometrically?

---

	cc. 0.2 M KH_2PO_4	cc. 0.2 M NaOH
5.8	50	3.66
6.0	50	5.64
6.2	50	8.55
6.4	50	12.60
6.6	50	17.74
6.8	50	23.60
7.0	50	29.54
7.2	50	34.90
7.4	50	39.34
7.6	50	42.74
7.8	50	45.17
8.0	50	46.85
	cc. 0.2 M Boric Acid	
8.2	50	5.90
8.4	50	8.55
8.6	50	12.00
8.8	50	16.40
9.0	50	21.40
9.2	50	26.70
9.4	50	32.00
9.6	50	36.85
9.8	50	40.80
10.0	50	43.90

From *The Determination of Hydrogen Ions* by W. M. Clark, Williams and Wilkins Company, Baltimore.

TABLE 1

COMMON INDICATORS

Indicator		Color		*p*H Range	Preparation for Use
Common Name	Chemical Name	Acid	Base		
Thymol blue	Thymolsulfonephthalein	Red	Yellow	1.2–2.8 (acid range) 8.0–9.6 (alkali range)	0.1% water solution of sodium salt
Methyl orange	Dimethylaminoazobenzenesulfonate	Red	Yellow	2.9–4.0	0.1% solution in 50% alcohol
Congo red	Sodium tetrazodiphenylnaphthionate	Blue	Red	3.0–5.0	0.5% alcoholic solution
Methyl red	*o*-Carboxybenzeneazodimethyl aniline	Red	Yellow	4.4–6.0	0.05% alcoholic solution
Alizarin red	Sodium alizarin sulfonate	Yellow	Purple	5.5–6.8	1.0% water solution of sodium salt
Bromothymol blue	Dibromothymolsulfonephthalein	Yellow	Blue	6.0–7.6	0.1% water solution of sodium salt
Litmus	....	Red	Blue	About 7.0	Paper
Cresol red	*o*-Cresolsulfonephthalein	Yellow	Red	7.2–8.8	0.1% solution in 50% alcohol
Phenolphthalein	Dihydroxyphthalophenone	Colorless	Red	8.3–10.0	1.0% solution in 50% alcohol
Cresolphthalein	*o*-Cresolphthalein	Colorless	Red	8.0–10.0	0.5% alcoholic solution

COLLOIDS

In order to secure good results in many of the experiments on colloids, special attention must be paid to cleanliness of apparatus. The glassware should be made of low-solubility glass and should be cleaned with hot cleaning fluid before using, care being taken to remove the last traces of cleaning fluid by thorough rinsing with distilled water. If these precautions are followed, good results may be expected.

Experiment 12

Preparation of a Ferric Hydroxide Sol

Place 300 cc. of distilled water in a clean beaker, and heat to boiling. Add to this, drop by drop, stirring constantly with a stirring rod, 10 per cent $FeCl_3$ solution. The result should be a clear $Fe(OH)_3$ sol of a medium brown color. If too much $FeCl_3$ solution is added, the color will become dark brown, indicating too high a concentration of $Fe(OH)_3$ and precipitation may occur during dialysis.

Since $FeCl_3$ hydrolyzes when added to water, forming $Fe(OH)_3$ and HCl, and since the HCl will interfere when the sol is used in a subsequent experiment, it is removed by dialysis. Prepare a collodion membrane as you did in Experiment 8, using a 500-cc. Erlenmeyer flask. Place the $Fe(OH)_3$ sol in the collodion membrane; tie a string around the neck of the membrane, and suspend it in a beaker of distilled water so that the level of the liquids is the same inside as outside. Allow dialysis to continue until the next laboratory period, changing the water in the beaker frequently. When dialysis is complete, transfer the sol to a clean flask or bottle for use in Experiment 15.

QUESTIONS

1. Is a $Fe(OH)_3$ sol a suspensoid or an emulsoid?
2. Are the colloidal particles positively or negatively charged?
3. By what method can the sign of the charge be determined?
4. Write an equation showing the hydrolysis of $FeCl_3$.

5. Give a theory accounting for the origin of the charge on the $Fe(OH)_3$ particles.
6. What is collodion?
7. What is removed during dialysis?

Experiment 13

Preparation of a Gold Sol

By means of a clean graduate transfer 230 cc. of distilled water to a clean 500-cc. Erlenmeyer flask. Place the flask on a wire gauze on a ring stand, and heat to boiling. Extinguish the flame, and add in rapid succession 4.2 cc. of 0.6 per cent gold chloride solution, 12.5 cc. of 1 per cent sodium citrate solution, and (slowly) 4.5 cc. of 0.1 per cent hydrogen peroxide solution. During the addition of the reagents the solution should be mixed vigorously by rotating the flask. The resulting gold sol should be old rose in color by reflected light, and a clear, deep red by transmitted light. Keep the gold sol for use in Experiments 14 and 15.

QUESTIONS

1. Is a gold sol an emulsoid or a suspensoid?
2. What is the sign of the charge on the gold particles?
3. What is the relative size of the particles in red and blue gold sols?

Experiment 14

Determination of the Gold Number of a 1 Per Cent Gelatin Sol

Thoroughly clean 10 test tubes and a 1-cc. and a 10-cc. graduated pipette. Place the test tubes in a test-tube rack; add to the first tube, by means of the 10-cc. graduated pipette, 9.9 cc. of gold sol. To each of the other tubes add 5 cc. of gold sol. By means of the 1-cc. graduated pipette add to the first tube 0.1 cc. of 1 per cent gelatin sol, which is equivalent to 1.0 mg. of gelatin.

Introduce the 10-cc. graduated pipette into this tube, and mix thoroughly by drawing the liquid into the pipette several times. Transfer 5 cc. of this mixture to the second tube, and mix as before. Transfer 5 cc. of the mixture in the second tube to the third tube, mix, and continue the process until the next to the last tube is reached. From this tube discard 5 cc. after mixing. Each tube will now contain 5 cc. of liquid, all except the last

containing gelatin. The last tube is a control. The amount of gelatin in any tube is one-half the amount in the preceding tube, the first tube containing 0.5 mg. of gelatin.

Now add 0.5 cc. of 10 per cent NaCl solution to each tube. Down to a certain point the gold sols should remain red. In the next tube and in all subsequent tubes the gold sol will appear blue or will be precipitated. Twice the number of milligrams of gelatin in the first tube which is blue is the gold number of the gelatin.

QUESTIONS

1. Define gold number.
2. What is meant by a protective colloid?
3. How many milligrams of gelatin are there in 0.1 cc. of 1 per cent gelatin sol?
4. How many milligrams of gelatin were left in the first tube after removing 5 cc.?
5. Which was the first tube that turned blue?
6. How many milligrams of gelatin were there in this tube?
7. What is the gold number of the gelatin?

Experiment 15

Mutual Precipitation of Sols

Place a series of 7 clean test tubes in a test-tube rack. Add to each tube gold sol and $Fe(OH)_3$ sol as follows:

Tube number	1	2	3	4	5	6	7
Gold sol, cc.	0.5	1.0	1.5	2.0	2.5	3.0	3.5
$Fe(OH)_3$ sol, cc.	3.5	3.0	2.5	2.0	1.5	1.0	0.5

Mix the contents of each tube thoroughly; allow the tubes to stand for an hour, and observe which ones show precipitation. The purpose of varying the proportions of the two sols in the above series is to facilitate securing a proportion of the sols which will give a complete neutralization of charge. One or more of the tubes may show precipitation.

QUESTIONS

1. Which tubes show precipitation?
2. Is it the gold or $Fe(OH)_3$ sol which precipitates, or do both precipitate?
3. Why is there precipitation?
4. If an excess of one sol is present in a tube, what happens to the sign of the charge on the other sol?
5. If there is a large excess of one sol, why do not the other sol and an equivalent amount of the sol in excess precipitate?

Experiment 16

Reversible Gelation

Obtain from the instructor a test tube containing a 1½ per cent agar-agar gel. Place the test tube in a beaker of water, and heat. Note the temperature of the water at which the gel becomes a sol. Now allow the beaker of water, with the test tube in it, to cool, and observe the temperature at which a gel is formed.

QUESTIONS

1. At what temperature does the agar-agar gel liquefy, and at what temperature does the agar-agar sol solidify?

2. What is the source of agar-agar?

3. Name an important use of agar-agar.

4. State a theory explaining what happens when a sol forms a gel.

5. What is an irreversible gelation called? Give an example.

Experiment 17

Adsorption

Place 20 cc. of caramel solution in an Erlenmeyer flask; add about 2 grams of powdered bone black, shake thoroughly, and filter. Compare the color of the filtrate with the original caramel solution.

QUESTIONS

1. What was the color of the caramel solution? Of the filtrate?

2. Define adsorption.

3. What is meant by negative adsorption?

4. In terms of their effect on surface tension, what substances in solution are adsorbed?

CARBOHYDRATES

Caution. Many of the tests which follow are performed in test tubes. Many of the reagents used in test-tube reactions are dangerous. Great care should be taken in heating solutions in test tubes over a free flame, because it is a common occurrence for a hot liquid to be shot out of the mouth of a test tube. Always make sure that the mouth of the test tube is not pointing toward your own face or that of your neighbor. The author has seen one student permanently blinded by nonobservance of this simple precaution.

Experiment 18*

Effect of Acids on Monosaccharides

Connect a 250-cc distilling flask to a condenser, and place in the flask 5 grams of wheat bran, 35 cc. of water, and 35 cc. of concentrated HCl. The wheat bran is used as a source of pentose. Distil about half the liquid, testing the vapor at the end of the condenser during the early part of the distillation with a piece of filter paper moistened with aniline acetate solution.[1] A color should appear on the paper. Disconnect the distilling flask, and filter the residue in the flask. Place the filtrate in a separatory funnel, add 15 cc. of ethyl ether, and shake. Draw off the filtrate, and place the ether in an evaporating dish. Repeat the extraction of the filtrate with ether, adding the ether to the evaporating dish. Evaporate the ether on a steam bath, and dissolve the residue in a little 5 per cent NaOH solution. Place in a test tube, and add iodine solution[2] until a tan color remains. Note the odor, and examine under the microscope any crystals formed. Repeat the experiment, using 5 grams of glucose in place of the bran.

[1] Aniline acetate solution: Mix equal volumes of aniline and water, and add glacial acetic acid to the mixture until a clear solution results on shaking.

[2] Iodine solution: Dissolve 60 grams of potassium iodide in about 100 cc. of water. Dissolve 40 grams of iodine in this solution, and dilute to 1 liter with water.

QUESTIONS

1. What happened to the aniline acetate in each test?
2. Were crystals of iodoform formed in each test?
3. Write an equation showing what happens to a pentose when boiled with HCl.
4. Is furfuraldehyde volatile?
5. What caused the aniline acetate to become colored?
6. What products are formed when a hexose is boiled with HCl?
7. Is levulinic acid volatile?
8. How may levulinic acid be tested for?

Experiment 19

General Test for Carbohydrates (Molisch's Test)

To 5 cc. of 5 per cent glucose solution in a test tube, add 1 drop of Molisch's reagent[3] and mix. Incline the tube, and add about 5 cc. of concentrated H_2SO_4, allowing the acid to run gently down the side of the tube. The H_2SO_4, having a greater specific gravity than the glucose solution, will form a layer at the bottom of the tube. Notice the color at the juncture of the two liquids.

QUESTIONS

1. What color appeared where the two layers meet?
2. Explain the production of the color.
3. What is the composition of Molisch's reagent?
4. Is this a specific test for glucose?

Experiment 20

Action of Strong Alkali on Carbohydrates (Moore's Test)

Place 5 cc. of 5 per cent glucose solution in one test tube and 5 cc. of 5 per cent sucrose in another. Add 1 cc. of 10 per cent NaOH solution to each test tube, and place the tubes in a beaker of boiling water for 5 minutes. Notice the color and odor of the solutions in the tubes.

QUESTIONS

1. What are the appearance and color of each tube?
2. What chemical change took place in the tube that developed a color?
3. Why does sucrose act differently from glucose?

---

[3] Molisch's reagent is made by dissolving 10 grams of α-naphthol in 100 cc. of 95 per cent ethyl alcohol.

Experiment 21

Effect of Organic Hydroxy Compounds on the Precipitation of Cupric Hydroxide

Place 4 test tubes in a test-tube rack, and add to each 2 cc. of 1 per cent $CuSO_4$ solution and 2 cc. of 10 per cent NaOH solution. To the second tube add 2 cc. of 5 per cent glucose solution, to the third 2 cc. of 30 per cent sodium citrate solution, and to the fourth 2 cc. of 30 per cent sodium potassium tartrate solution (Rochelle salt). Mix the contents of each tube; place the tubes in a beaker of boiling water, and heat for 2 minutes. Observe the results.

QUESTIONS

1. What happens in each tube?
2. Write an equation showing what happens when NaOH is added to $CuSO_4$.
3. Write an equation showing what happens in the fourth tube.
4. What are the color, name, and formula for the precipitate formed in the second tube on heating?
5. Explain what has happened in the second tube.

Experiment 22

Reduction Tests for Carbohydrates

1. **Fehling's Test.** Mix ½-cc. portions of Fehling's solutions A and B[4] in a test tube and dilute with 4 cc. of water. Heat to boiling, and add a few drops of a 5 per cent glucose solution, continuing the boiling. Repeat the experiment, using 5 per cent solutions of lactose and sucrose.

2. **Benedict's Qualitative Test.** To 5 cc. of Benedict's qualitative reagent[5] in a test tube add ½ cc. of 5 per cent glucose solution. Place in a beaker of boiling water for 2 minutes, and allow

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[4] Fehling's solution A: Dissolve 69.38 grams of copper sulfate in water and make up to a volume of 1 liter.

Fehling's solution B: Dissolve 250 grams of NaOH in water, add 346 grams of sodium potassium tartrate, and make up to a volume of 1 liter.

[5] Benedict's qualitative reagent: Dissolve 173 grams of crystalline sodium citrate and 100 grams of anhydrous Na_2CO_3 in 800 cc. of water. Filter, and add a filtered solution of 17.3 grams of copper sulfate in 100 cc. of water. Make the solution up to a volume of 1 liter.

to cool in the air. Repeat the experiment, using 5 per cent solutions of lactose and sucrose.

3. Nylander's Test. Place 5 cc of 5 per cent glucose solution and ½ cc. of Nylander's reagent[6] in a test tube, and heat in a beaker of boiling water. Repeat the experiment, using 5 per cent solutions of lactose and sucrose.

4. Barfoed's Test. Place 5 cc. of Barfoed's reagent[7] in a test tube, and add ½ cc. of 5 per cent glucose solution. Place in a beaker of boiling water, and continue heating for several minutes. Repeat the test, using 5 per cent solutions of lactose and sucrose. Note the length of time required for reduction to take place and also the relative amounts of precipitates.

5. Picric Acid. To 5 cc. of 5 per cent glucose solution in a test tube add 3 cc. of saturated picric acid solution and 1 cc. of 10 per cent NaOH solution. Place in a beaker of boiling water.

6. Phosphotungstic Acid. To 5 cc. of 5 per cent glucose solution in a test tube add 6 drops of 0.1 N NaOH solution, and heat to boiling. Add 3 drops of phosphotungstic acid solution.[8]

7. Potassium Permanganate. To 1 cc. of 5 per cent glucose solution add 2 drops of 10 per cent H_2SO_4 solution and a few drops of 0.05 N $KMnO_4$ solution. If no color change occurs, heat to boiling.

8. Silver Salts. Place in a test tube 1 cc. of 1 per cent $AgNO_3$ solution, 1 cc. of 10 per cent NH_4OH solution, and 1 cc. of 5 per cent glucose solution. Warm gently and set aside.

9. Methylene Blue. To 1 cc. of 5 per cent glucose solution in a test tube add 2 drops of 0.1 per cent methylene blue solution and 3 drops of 10 per cent NaOH solution. Heat in a beaker of boiling water for several minutes.

QUESTIONS

1a. What happened in each test?

b. How do you account for the difference in action of lactose and sucrose?

c. What is the formula for the precipitate?

[6] Nylander's reagent: Dissolve 20 grams of bismuth subnitrate and 40 grams of sodium potassium tartrate in 1 liter of hot 10 per cent KOH solution. Cool and filter.

[7] Barfoed's reagent: Dissolve 90 grams of crystallized cupric acetate in 1 liter of water, and add 6.0 cc. of glacial acetic acid. Filter.

[8] Phosphotungstic acid: Dissolve 100 grams of sodium tungstate and 70 grams of disodium phosphate in 500 cc. of water, and acidify with HNO_3.

d. State the composition of Fehling's solution and the function of each constituent.

e. Why are the two Fehling's solutions kept separate?

2a. What happened in each test?

b. What is the composition of Benedict's qualitative reagent?

c. What do you consider the main difference between Fehling's and Benedict's reagents?

d. Which reagent will detect smaller traces of sugar? Why?

3a. What happened in each test?

b. What is the precipitate formed?

c. What is the composition of Nylander's reagent?

d. Write equations showing what happens when Nylander's reagent is reduced.

4a. What happened in each test?

b. What was the relative time required for the reduction by glucose and lactose?

c. What were the relative volumes of precipitates formed with glucose and lactose?

d. What is the composition of Barfoed's reagent?

e. What do you consider the main difference between this and Fehling's solution?

5a. What was the color of the solution after boiling?

b. What are the name and formula for the colored compound?

6a. What was the color after heating with NaOH?

b. What was the color after adding phosphotungstic acid?

c. What is the probable formula for the final colored compound?

7. What happened to the $KMnO_4$ solution? Explain.

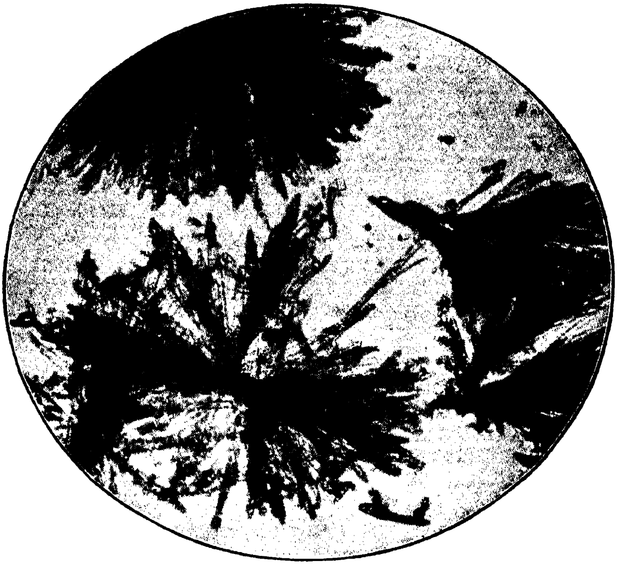
8. What happened on standing? Explain.

9. What happened on heating? Explain.

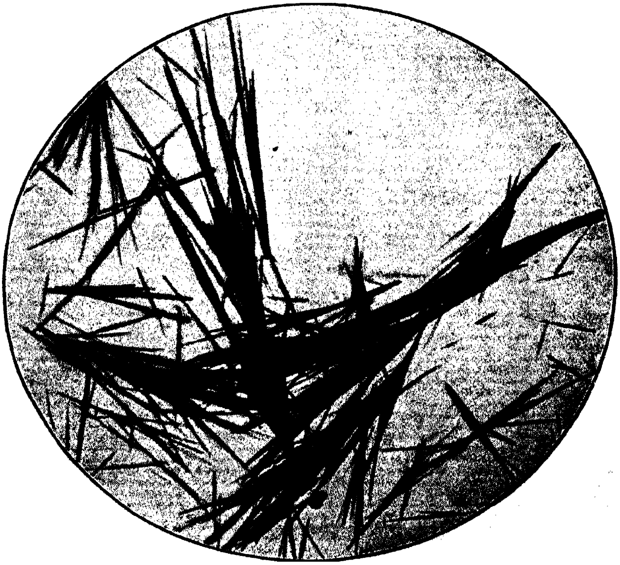
Experiment 23

Preparation of Osazones

Prepare 8 test tubes, placing 4 cc. of a 5 per cent sugar solution in each tube. Use the following sugars: xylose, glucose, fructose, mannose, galactose, maltose, lactose, and sucrose. Add to each tube 5 drops of phenylhydrazine and ½ cc. of glacial acetic acid. With constant shaking, heat over a low flame for 1 minute. Add about 6 drops of 10 per cent NaOH solution to each tube. Each solution should be faintly acid to litmus at this point. Heat in a beaker of boiling water for 30 to 40 minutes. When the tubes are allowed to cool gradually, yellow crystals of osazones should appear. Transfer some of the crystals from each tube to a

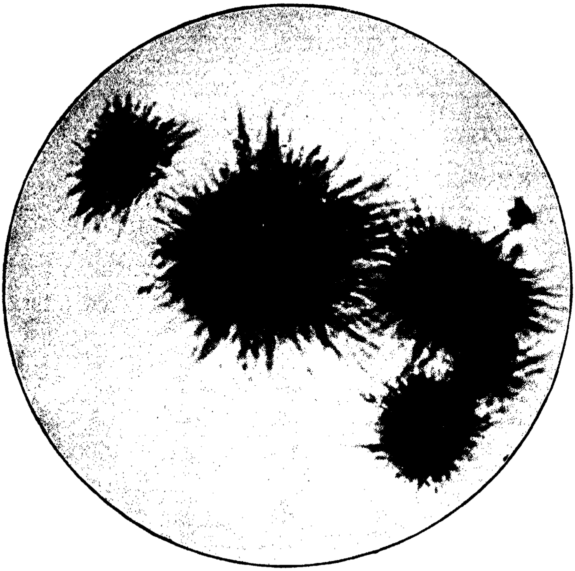


Galactosazone

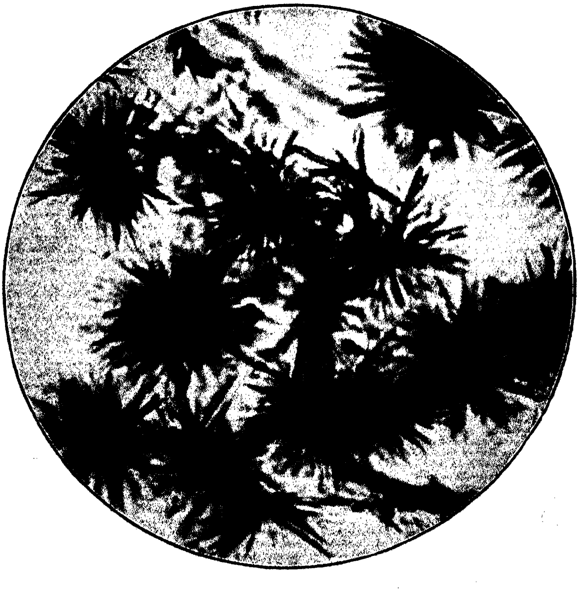


Glucosazone

FIG. 9. Osazones of common sugars. From *Biochemical Laboratory Methods* by Morrow and Sandstrom.



Lactosazone



Maltosazone
FIG. 9 (*continued*)

microscope slide by means of a stirring rod, cover with a cover slip, and examine the crystals under the low power of the microscope. Make sketches of the various osazone crystals. (See Fig. 9.)

QUESTIONS

1. Write equations showing the formation of glucosazone.
2. What three sugars gave the same osazone? Why?
3. Did sucrose give an osazone? Explain.
4. How did the reaction with mannose differ from that of the other sugars? Explain.

Experiment 24

Alcoholic Fermentation

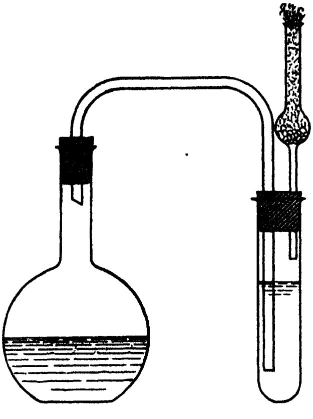


Fig. 10. Alcoholic fermentation apparatus.

1. Place 300 cc. of 10 per cent glucose solution in a 500-cc. flask, and add about one-quarter cake of compressed yeast broken into fine pieces. (See **2.**) Shake to make a uniform suspension of the yeast. Fit the flask with a one-hole rubber stopper through which passes a glass tube bent so that it extends to the bottom of a large test tube. (See Fig. 10.) The test tube is fitted with a two-hole rubber stopper. Through one hole is passed the glass tube from the flask, the other hole being fitted with a small soda lime tube. Fill about one-half of the test tube with saturated $Ba(OH)_2$ solution, and make certain that both stoppers of the apparatus are tight. Set the apparatus aside until the next laboratory period, at which time disconnect the apparatus, and wash the precipitate in the test tube with water by decantation. Add a little HCl to the precipitate, and note the effervescence. Connect the fermentation flask to a condenser, and distil about 50 cc. Test the distillate for ethyl alcohol by making 10 cc. of the distillate alkaline with NaOH solution and adding iodine solution, drop by drop, until a tan color persists. Heat gently; note the odor and the appearance of crystals under the microscope.

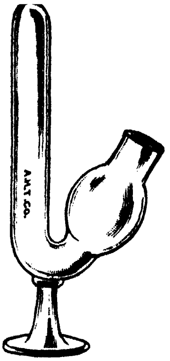


FIG. 11. Einhorn saccharometer.

2. Place in an Einhorn saccharometer (see Fig. 11) some of the glucose-yeast mixture used in the preceding experiment, using enough to fill the closed arm. The saccharometer should not be completely filled; if it is, it will run over as fermentation proceeds. Set aside until the next laboratory period, at which time estimate the volume of the gas in the closed arm. Add to the saccharometer a few drops of 50 per cent NaOH solution, and completely fill the bulb with water. Place your thumb over the mouth of the tube, and shake. Before removing your thumb make sure that all the gas is in the closed arm of the saccharometer. Remove your thumb, and estimate again the volume of gas in the closed arm.

QUESTIONS

1a. Write an equation showing what happens during the alcoholic fermentation of glucose.

b. What is the purpose of the soda lime tube?

c. What happens to the $Ba(OH)_2$? Write the equation.

d. What happens when the precipitate is treated with HCl? Write the equation.

e. Make a sketch of an iodoform crystal.

f. Is the iodoform test a specific test for ethyl alcohol?

g. What are the odor and color of iodoform?

h. Write the formula for iodoform.

2a. What is the approximate volume of the gas in the closed arm of the Einhorn saccharometer before and after treatment with NaOH solution?

b. Write an equation representing what happens to the gas when it is treated with NaOH solution.

Experiment 25*

Mutarotation

Dissolve about 10 grams of glucose in 100 cc. of water. Divide the solution into two parts; to one part add a drop of NH_4OH solution, and mix. Fill a polariscope tube with the other part, and read immediately in a polariscope[9] or saccharimeter. Return the solution in the polariscope tube to the flask containing the

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[9] Obtain directions for the operation of the polariscope or saccharimeter from the instructor.

rest of this solution, and fill the tube with the solution containing NH_4OH; read in the polariscope. Now discard the solution in the polariscope tube, and rinse the tube first with water and then with a few cubic centimeters of the sugar solution originally in it. Fill the tube with this solution, and read again in the polariscope. This reading should be made 30 minutes after the original reading. Retaining the solution in the polariscope tube, read at the end of an hour and again at the end of the laboratory period, recording the time elapsed between the first and last reading.

QUESTIONS

1. What are the various readings of the glucose solution containing no NH_4OH?
2. What is the reading of the solution containing NH_4OH?
3. Was the original sugar α- or *β-d*-glucose?
4. Explain the mutarotation of glucose.
5. What structure must be present in a sugar molecule for it to show mutarotation?
6. What is the difference between a polariscope and a saccharimeter?
7. What structure in a molecule makes a compound optically active?

Experiment 26

Seliwanoff's Test for Fructose

Place 5 cc. of Seliwanoff's reagent[10] in a test tube, and add 1 cc. of 5 per cent fructose solution. Immerse in a beaker of boiling water until a precipitate forms. Filter, and dissolve the precipitate in ethyl alcohol.

QUESTIONS

1. What happened on boiling?
2. What is the color of the alcohol solution?
3. What kind of sugar is fructose?
4. Is Seliwanoff's test a specific test for fructose?
5. What is the composition of Seliwanoff's reagent?
6. Does fructose reduce Fehling's and Barfoed's solutions?

Experiment 27

Mucic Acid Test for Galactose

Place 50 cc. of 5 per cent galactose solution in a small beaker, and add 5 cc. of concentrated HNO_3. Evaporate to about 10 cc.

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[10] Seliwanoff's reagent: Dissolve 1.5 grams of resorcinol in 1 liter of dilute HCl (1 part concentrated HCl to 2 parts water).

on a steam bath, cool, and allow to stand until the next laboratory period. Note the appearance of any crystals formed. Repeat the test on a 5 per cent lactose solution.

QUESTIONS

1. What happened on standing?
2. What was the color of the crystals?
3. Write the graphic formula for mucic acid.
4. How is mucic acid related chemically to galactose?
5. Does galactose reduce Fehling's and Barfoed's solutions?
6. What does lactose give on hydrolysis?
7. What is β-lactose?

Experiment 28*

Specific Rotation and Inversion of Sucrose

1. Specific Rotation. Carefully weigh about a 10-gram sample of sucrose, and transfer it to a 100-cc. graduated flask. Dissolve in water, make up to volume, and mix thoroughly. If the polariscope tube is wet, rinse it out with a little of the sucrose solution, and then fill the tube, sliding the glass cover onto the end of the tube to avoid air bubbles. Place the tube in a polariscope and read. Calculate the specific rotation of sucrose by the following formula:

$$\text{Specific rotation} = \frac{\text{Observed reading}}{\text{Grams sucrose in 1 cc.} \times \text{Length of tube in decimeters}}$$

If a saccharimeter is used in place of a polariscope, multiply the observed reading by the factor 0.3466 to convert saccharimeter readings into angular degrees.

2. Inversion. Transfer by means of a pipette 50 cc. of your sucrose solution to a 100-cc. graduated flask; add 25 cc. of water and 5 cc. of concentrated HCl. Mix thoroughly, place a thermometer in the flask, and immerse the flask in a beaker of water heated to 85°C. When the temperature in the flask reaches 69°C., transfer the flask to another beaker of water whose temperature is 68° to 70°C., maintaining the temperature until the total time of heating is 10 minutes. Cool to 20°C., rinse the thermometer with water into the flask, and make up to volume.

Mix thoroughly, and read in the polariscope. Since the sucrose solution has had its volume doubled, the reading must be multiplied by 2 to give the invert reading on the undiluted sample. From the readings before and after inversion, calculate the percentage of sucrose in the sample. This should equal the amount of sucrose originally weighed out. For this calculation the following formula, based on the fact that 26 grams of sucrose in 100 cc. gives a positive reading (P) of +34.66° before inversion, and an invert reading (I) of −11.32° after inversion, the difference in reading being 45.98°, is used.

$$45.98 : P\text{-}I :: 26 : x$$

$$x = \text{grams sucrose per 100 cc.}$$

If a saccharimeter is used, the positive reading is +100° and the invert reading is −32.66°, the difference being 132.66°. The formula then becomes

$$132.66 : P\text{-}I :: 26 : x$$

It should be noted that I is a negative quantity and that P-I is an algebraic difference.

After this work has been completed, neutralize the remaining invert sugar solution with 10 per cent NaOH, and test with Fehling's and Barfoed's solution.

QUESTIONS

1a. Define specific rotation.

b. What are the specific rotations of the following sugars: glucose, fructose, galactose, mannose, maltose, lactose, and sucrose?

c. In determining the specific rotation of glucose, how would you avoid an error due to mutarotation?

d. What chemical structure is associated with optical activity?

e. What was the weight of sucrose taken for analysis?

f. How long was the polariscope tube?

g. What was the polariscope reading?

h. Give calculations for determining the specific rotation of sucrose.

2a. Why must care be taken in heating the sucrose solution during inversion?

b. Why must care be taken to make the invert reading at 20°C.?

c. Why must the invert reading be multiplied by 2?

d. Give calculations for determining the percentage of sucrose in the original solution.

Experiment 29

Preparation and Properties of Starch

1. Preparation. Pare a small potato, and reduce it to a fine pulp on a grater. Place the pulp in a beaker, and mix well with water. Strain the pulp through a piece of cheesecloth, catching the liquid in a beaker. Allow the starch to settle, and pour off the supernatant liquid. Wash the starch several times by decantation. Allow the starch to drain thoroughly, and set aside to dry.

2. Properties. Place about 1 gram of starch in a test tube of cold water; shake to form a suspension. Pour this suspension slowly into 100 cc. of boiling water in a beaker, stirring constantly. Cool, and test a portion of this solution in a test tube with a drop of iodine solution.[2] Heat the blue solution in the test tube to boiling, and cool. Test a few drops of the starch solution with Fehling's solution.

Place 5 cc. of the starch solution in a test tube, and add 1 cc. of 50 per cent NaOH solution. Heat to boiling, and continue heating for 2 minutes. Cool, and just neutralize with HCl solution. Test some of this solution with Fehling's solution.

To the remaining starch solution add 5 cc. of concentrated HCl, and heat to boiling. Continue boiling, maintaining the original volume by the addition of water from time to time. To each depression on a spot plate add one drop of iodine solution, and at frequent intervals, by means of a stirring rod, transfer a drop of the boiling starch solution to a depression on the spot plate. Continue the testing until the starch solution no longer gives a color with iodine, noting the time required to reach the achromatic point. Neutralize the residue with 10 per cent NaOH solution, and test it with Fehling's solution.

QUESTIONS

1. What is meant by washing by decantation?

2a. What was the color when iodine was added to starch?

b. What happened on boiling? On cooling?

c. Could starch be used as an indicator in titrating iodine if the solution were hot?

d. Does starch reduce Fehling's solution?

e. Does starch give a positive Moore's test?

f. Is starch hydrolyzed by NaOH?

g. Is Fehling's test positive after boiling with NaOH?
h. Is starch more stable in acid or alkaline solution?
i. Is glucose more stable in acid or alkaline solution?
j. What happens to starch on boiling with HCl?
k. What does starch give on complete hydrolysis?
l. How long did it take the starch to reach the achromatic point?
m. Why must the solution be neutralized before testing with Fehling's solution?
n. What was the result in the final Fehling's test?

Experiment 30

Identification of Starch Granules

Examine potato, arrowroot, corn, rice, and wheat starch under the microscope. This is best done by moistening a small pinch of each of the various starches on a microscope slide with a drop of water before making the examination. Make sketches of the various kinds of starch granules. Obtain an unknown sample of starch from the instructor, and identify it by microscopic examination. (See Fig. 12.)

QUESTIONS

1. Make sketches of the various kinds of starch granules.
2. What kind of starch was your unknown sample?

Experiment 31

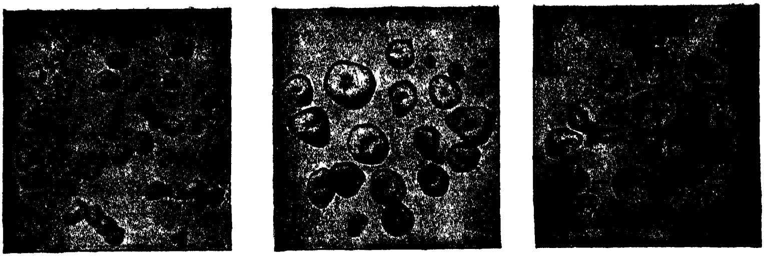
Dextrin

1. Make a Fehling's test on some 2 per cent dextrin solution.

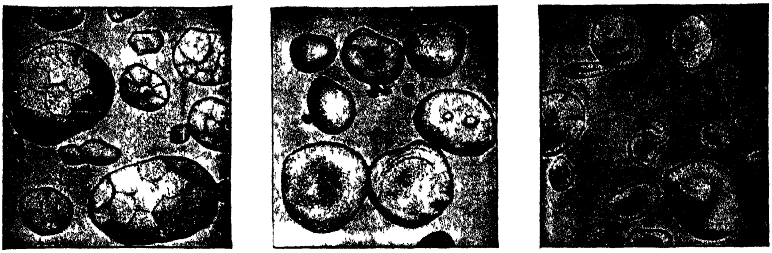
2. To 5 cc. of 2 per cent dextrin solution, add an equal volume of 95 per cent ethyl alcohol.

3. To 10 cc. of 2 per cent dextrin solution add an equal volume of saturated $(NH_4)_2SO_4$ solution. Shake, allow to stand for 5 minutes, and filter. The $(NH_4)_2SO_4$ will precipitate starch, which is usually present in dextrin. Test a few drops of the filtrate with iodine solution.[2]

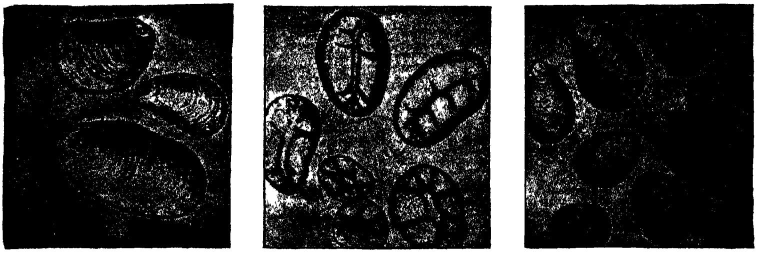
4. To the remaining filtrate add 2 drops of concentrated HCl, and heat to boiling. Continue the boiling, maintaining the original volume by frequent additions of water, and by means of a spot plate and iodine solution determine the time required to reach the achromatic point.



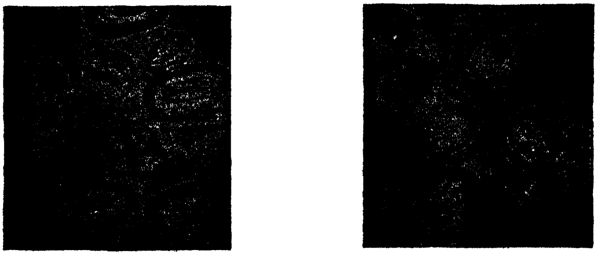
Buckwheat Maize Rice



Oat Barley Rye



Potato Bean Arrowroot



Pea Wheat

Fig 12 Starch granules from various sources (after Leffmann and Beam) From *Practical Physiological Chemistry* by Hawk, Oser and Summerson. Courtesy, P Blakiston's Son and Co.

QUESTIONS

1. What was the result with Fehling's test?
2. What happened when alcohol was added to the dextrin solution?
3a. Was there any starch in the dextrin solution?
  b. Why would starch interfere with the iodine test?
4a. How long did it take to reach the achromatic point?
  b. What is the difference between erythrodextrin and achroödextrin?

Experiment 32*

Preparation and Properties of Glycogen

1. Preparation. Cut a fresh oyster into small pieces; mix them with sand, and grind in a mortar. Transfer the mixture to a beaker, add 25 cc. of water, and heat to boiling for 20 minutes, adding water from time to time to maintain the original volume. Make faintly acid to litmus with 5 per cent acetic acid solution, boil for 1 minute, cool, and filter. Add to the filtrate 4 volumes of 95 per cent alcohol. Allow the precipitate to settle, pour off the supernatant liquid, and filter. Dissolve the precipitate in 15 cc. of hot water.

2. Properties. a. Test a few drops of the glycogen solution with Fehling's solution.

b. To 5 cc. of the glycogen solution add 5 drops of iodine solution[2] and 3 drops of 10 per cent NaCl solution.

c. To the remainder of the glycogen solution add about ½ cc. of concentrated HCl, and boil for 10 minutes. Cool, neutralize with 10 per cent NaOH solution, and make a Fehling's test.

QUESTIONS

1. What is another good source of glycogen?
2. What was the result of the Fehling's test before hydrolysis?
3. What was the color when glycogen was treated with iodine?
4. What does glycogen give on hydrolysis?
5. What was the result with Fehling's test after hydrolysis?

Experiment 33

Cellulose

1. Place a little cotton in a test tube containing Schweitzer's

reagent.[11] Stir with a glass rod until the cotton is dissolved. Acidify with glacial acetic acid.

2. Place a little cotton in a test tube containing Cross and Bevan's reagent.[12] Stir with a glass rod until the cotton is dissolved. Add an equal volume of 95 per cent alcohol.

3. Mix 5 cc. of dilute and 10 cc. of concentrated H_2SO_4 in a small beaker. Cool, add a little cotton, and stir with a glass rod. When the cotton has completely dissolved, allow to stand for 10 minutes, then dilute with an equal volume of water, and boil gently for 15 minutes, maintaining the original volume by frequent additions of water. Cool, and neutralize with 50 per cent NaOH solution (caution). Test with Fehling's solution.

QUESTIONS

1a. How is Schweitzer's reagent made?
  b. What happens to the cotton in Schweitzer's reagent?
  c. What happens on acidifying with acetic acid?
2a. What is the composition of Cross and Bevan's reagent?
  b. What happens to the cotton in Cross and Bevan's reagent?
  c. What happens when alcohol is added?
3a. What does normal cellulose give on complete hydrolysis?
  b. What happens on boiling with acid?
  c. What was the result with Fehling's test?

Experiment 34

Inulin

Make the following tests on a 2 per cent solution of inulin:

1. Molisch's test.

2. Fehling's test.

3. Add a drop of iodine solution[2] to 2 cc. of inulin solution in a test tube.

4. Add 2 drops of concentrated HCl to 5 cc. of inulin solution,

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[11] Schweitzer's reagent: To 100 grams of copper turnings in a wide-mouthed bottle add 1 liter of concentrated ammonium hydroxide. Allow to stand without a stopper, with frequent agitation, for 24 hours or until a deep blue solution results. Insert a stopper through which passes a glass tube. Attach to the glass tube a rubber tube closed at the other end by means of a glass rod. This rubber tube has a short slit in it to allow gas to pass out, but not in. Pour off the clear supernatant liquid in quantities for immediate use. The solution deteriorates rapidly when allowed to stand in a reagent bottle.

[12] Cross and Bevan's reagent: Dissolve 60 grams of zinc chloride in 100 cc. of concentrated HCl.

and heat to boiling for 1 minute. Cool, neutralize with 50 per cent NaOH solution, and make a Fehling's test on some of this solution. Place 1 cc. of the remaining solution in a test tube and add 5 cc. of Seliwanoff's reagent.[10] Place the test tube in a beaker of boiling water and note what happens.

QUESTIONS

1a. What was the result of Molisch's test?
 b. What does this result tell about inulin?
2. Does inulin reduce Fehling's solution?
3. What was the color when iodine was added?
4a. What was the result of the Fehling's test after boiling with HCl?
 b. What does inulin give on hydrolysis?
 c. What was the result of Seliwanoff's test?

Experiment 35

Agar-Agar

1. Test the solubility of agar-agar in cold and hot water. Cool the hot water solution.

2. Place about 0.2 gram of agar-agar in a small beaker; add 10 cc. of water and 10 cc. of concentrated HCl. Boil for 10 minutes, maintaining the original volume by frequent additions of water. Cool, and neutralize with 50 per cent NaOH solution. If necessary, clarify the solution by adding animal charcoal and filtering. Test a portion of this filtrate with Fehling's solution, and make a mucic acid test on what remains. (See Experiment 27.)

QUESTIONS

1a. What is the solubility of agar-agar in cold and hot water?
 b. What happened when the hot solution cooled?
 c. How is agar-agar used in bacteriology?
2a. Did the solution solidify on cooling? Why?
 b. What was the result of Fehling's test?
 c. Was the mucic acid test positive or negative?
 d. What does agar-agar give on hydrolysis?

Experiment 36

Pentosans

To a small piece of gum arabic in a large test tube add 5 cc. of water and 5 cc. of concentrated HCl. Boil for 10 minutes,

maintaining the original volume by frequent additions of water. Test the fumes given off with moist aniline acetate paper.[1] Cool, and neutralize with 50 per cent NaOH solution. If the solution is colored, clarify with animal charcoal and filter. Test the solution with Fehling's reagent.

QUESTIONS

1. What was the result with aniline acetate paper?
2. What does this result tell about the composition of gum arabic?
3. What was the result of Fehling's test?
4. What does gum arabic give on hydrolysis?

Experiment 37

Unknown Carbohydrate

Obtain an unknown carbohydrate from the instructor, and by using the foregoing tests determine which carbohydrate you have.

QUESTIONS

1. What carbohydrate did you find in your unknown sample?
2. What tests did you apply, and what was the result of each test?
3. On what basis did you finally identify your carbohydrate?

Experiment 38

Preparation and Properties of Pectin

1. Preparation. Remove the yellow layer from a grapefruit rind, and pass the white inner rind through a food chopper. Place the ground material in a beaker, and cover with distilled water. Allow the mixture to stand over night or until the next laboratory period. Drain the pulp through a cheesecloth, squeezing thoroughly. Replace the pulp in a beaker, cover with water, and boil for 2 hours, maintaining the volume of the liquid by frequent additions of water. At the end of 2 hours evaporate to a volume just sufficient to cover the pulp. Filter through cheesecloth. Cool the filtrate, and add twice its volume of 95 per cent alcohol. Filter, and dry the precipitated pectin.

2. Properties. Weigh a beaker on a rough balance, and add to this weighed beaker 1 gram of dried pectin, 20 grams of sucrose, 0.65 gram of citric acid, and 65 cc. of distilled water. Dissolve

by heating, and evaporate until the weight of the material in the beaker is 65 grams. Set away to cool over night.

QUESTIONS

1a. Name another good source of pectin.
b. Explain what happened when the pulp was boiled with water.
c. What is protopectin?
2a. What happened on cooling the solution?
b. What must be added to pectin to make a jelly?

LIPIDS

Experiment 39

Oils and Surface Tension

1. Clean a 1-cc. pipette thoroughly with cleaning fluid, and rinse thoroughly. Fill the pipette with distilled water and then slowly empty it, counting the number of drops. Dry the pipette, fill it with olive oil, and count the number of drops. Without washing the pipette fill it with water again, and count the number of drops.

2. Fill a clean beaker with distilled water, and sprinkle a few small pieces of camphor on the surface of the water. Observe the action of the camphor. Rub a clean glass rod against the nose, and touch it to the surface of the water.

QUESTIONS

1a. What determines the size of a drop of liquid?

b. What were the number of drops of water, oil, and water from the oily pipette?

c. Which has the greater surface tension, oil or water?

2a. Describe the action of the camphor on water.

b. What happened when the glass rod was touched to the water? Explain.

Experiment 40

Emulsification

Prepare 6 test tubes as follows:

TUBE

1. 5 cc. of water and 5 drops of neutral corn oil.[1]

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[1] Neutral corn oil: Place 100 cc. of corn oil in an Erlenmeyer flask, and add 50 cc. of 95 per cent alcohol and a few drops of phenolphthalein. Heat to boiling on a steam bath, and add 0.1 *N* NaOH until the alcohol layer remains pink after vigorous shaking. Remove the alcohol by washing several times with water in a separatory funnel. Finally separate the oily layer.

2. 5 cc. of water and 5 drops of rancid corn oil.[2]
3. 5 cc. of 0.02 *N* NaOH and 5 drops of neutral corn oil.
4. 5 cc. of 0.02 *N* NaOH and 5 drops of rancid corn oil.
5. 5 cc. of 0.1 per cent soap solution and 5 drops of neutral corn oil.
6. 5 cc. of 0.1 per cent soap solution and 5 drops of rancid corn oil.

Shake each tube thoroughly, and observe at once and at the end of 30 minutes.

QUESTIONS

1. What happened in each tube?
2. What are the two most important factors causing rancidity?
3. Is rancid oil acidic, basic, or neutral in reaction?
4. What does NaOH do to rancid oil?
5. How would you suggest making a kerosene emulsion for spraying purposes?

Experiment 41

Saponification of Fat

1. **Saponification.** Place 10 grams of lard in a 250-cc. Erlenmeyer flask, and add 50 cc. of 95 per cent alcohol and 7 cc. of 50 per cent NaOH solution. Fit the flask with a one-hole rubber stopper through which is passed a glass tube about 3 ft. long to serve as an air condenser. Heat on a steam bath for 1 hour. Transfer to an evaporating dish, and remove the alcohol by heating on a steam bath. When nearly all the liquid is evaporated, add 5 cc. of distilled water, and continue the evaporation. When the material in the dish is fairly dry, dissolve it in 500 cc. of distilled water.

2. **Separation of Soap.** To 10 cc. of your solution from **1** add 50 cc. of distilled water, and saturate with solid sodium chloride. Filter, and dissolve the precipitate in distilled water. Test portions of this solution by adding a few drops of 10 per cent calcium chloride, magnesium sulfate, and lead acetate solutions.

3. **Separation of Fatty Acids.** To the remaining 490 cc. of the solution you made in **1,** add concentrated HCl in sufficient quantity to liberate all the fatty acids from their salts. At this point heat until an oily layer rises to the surface. Cool until the

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[2] **Rancid corn oil: Add 1 cc. of oleic acid to 100 cc. of corn oil.**

top layer solidifies, and transfer the solid fatty acid layer to a beaker. (See **4.**) Add 100 cc. of distilled water, heat until the fatty acids melt, and stir to wash the fatty acids. Cool, remove the solid fatty acid layer, and dry between filter papers. Weigh accurately about 1 gram of the dry fatty acids on a small watch glass, transfer the watch glass with the fatty acid on it to a beaker, and add 25 cc. of 95 per cent alcohol. Add a few drops of phenolphthalein and heat to boiling. Titrate with 0.1 *N* NaOH solution to a permanent pink color, and calculate the number of cubic centimeters of 0.1 *N* NaOH solution necessary to neutralize 1 gram of the fatty acids.

Determine the melting point of the fatty acids as follows: Prepare a melting-point tube by heating the center of a heavy-walled glass tube about 1 cm. in diameter until the glass is soft over a length of about 2 in. A wing-tip burner is good for this purpose. Quickly draw out the glass tube until it is about 2 mm. in diameter. Cool, and cut the fine tube into lengths of about 2 in. for use as melting-point tubes.

Melt some of the fatty acids, and draw them into a melting-point tube. There should be a column of fatty acids about 1 cm. long in the tube. Draw the fatty acids up the tube to within a few millimeters of the top, and introduce the top end of the tube into the edge of the flame to seal it. Place a rubber band around the lower end of a thermometer to hold the melting-point tube in place so that the fatty acid column is next to the bulb of the thermometer. (See Fig. 13.) Support the thermometer in a beaker of water, avoiding covering the melting-point tube, and heat gradually. Note the temperature at which the fatty acids melt.

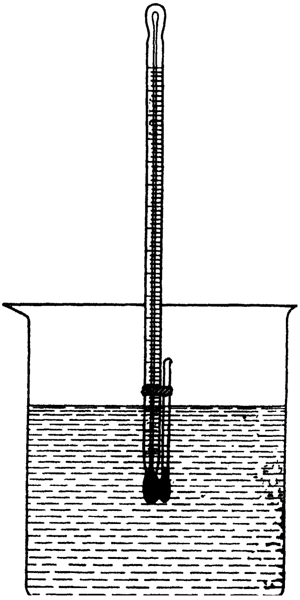

Fig. 13. **Melting-point apparatus.**

4. Separation of Glycerol. After the removal of the solid fatty acids neutralize the aqueous layer with 10 per cent NaOH solution, using litmus paper as an indicator. Evaporate over a free flame

to a small volume and finally to dryness on a steam bath. Extract twice with 25-cc. portions of 95 per cent alcohol, filtering each time into a small beaker. Evaporate the filtrate to a syrup on a steam bath. Test this material for glycerol as indicated in **5.**

5. Tests for Glycerol. a. Acrolein Test. Place 1 gram of $KHSO_4$ and 3 drops of glycerol in a crucible, and heat over a free flame, avoiding charring. Note the odor.

b. Flame Test. Fuse a little borax on a loop of a platinum or nichrome wire, and dip in glycerol. Heat in the flame again, noting the color of the flame.

QUESTIONS

1a. Assuming the fat to be tristearin, write an equation representing the reaction taking place during saponification.

b. Why is alcoholic NaOH used for saponification?

2a. Assuming the soap to be a hydrophilic colloid, explain its precipitation by NaCl.

b. What happened when $CuCl_2$, $MgSO_4$, and $Pb(C_2H_3O_2)_2$ solutions were added to the soap solution?

3a. Assuming the soap to be sodium stearate, write an equation representing the reaction when HCl was added to the soap solution.

b. What weight of fatty acids did you use for titration?

c. What was the normality factor of the NaOH solution used for titration?

d. How many cubic centimeters of this solution were used to neutralize the fatty acids?

e. To how many cubic centimeters of 0.1 *N* NaOH solution is this equivalent?

f. How many cubic centimeters of 0.1 *N* NaOH solution would be required to neutralize 1 gram of fatty acids?

g. What is the melting point of the fatty acids?

4. Write the formula for glycerol.

5a. Write an equation representing what happens when glycerol is heated with $KHSO_4$.

b. Describe the odor of acrolein.

c. What was the color of the flame in the borax flame test?

Experiment 42*

Volatile Fatty Acids in Butterfat

Place about 5 grams of butterfat[3] in a 250-cc. flask. Add

[3] Butterfat may be prepared by melting butter and then allowing it to stand until it separates into two layers. Filter the top butterfat layer through a hot water funnel.

2 cc. of 50 per cent NaOH solution and 10 cc. of 95 per cent alcohol. Connect the flask to a reflux air condenser, and heat on a steam bath for 25 minutes. Remove the condenser, and evaporate the alcohol on a steam bath. Add 140 cc. of recently boiled distilled water, and warm until a clear solution is obtained. Cool, and add 3 cc. of concentrated H_2SO_4. Add a few small pieces of pumice to prevent bumping, attach the flask to a water condenser, and distil about 100 cc. Notice the odor of the distillate. Filter the distillate, washing the filter with recently boiled distilled water. Titrate the filtrate with 0.1 *N* NaOH solution, using phenolphthalein as an indicator. Place the filter paper, containing the insoluble volatile fatty acids, in a beaker, and add 25 cc. of neutral 95 per cent alcohol. Add phenolphthalein, and titrate with 0.1 *N* NaOH solution.

QUESTIONS

1. Name the volatile fatty acids.
2. Of what is the oily layer remaining in the distilling flask composed?
3. Why is recently boiled distilled water used?
4. Define the Reichert-Meissl number.
5. Define the Polenske number.
6. How can you distinguish between cocoanut oil and butterfat?
7. Describe the odor of the volatile fatty acids.

Experiment 43 *

Drying of Oils

Place 5 drops of olive, cottonseed, and linseed oil each on separate small watch glasses, weigh each watch glass with its oil on an analytical balance. Place the watch glasses on a piece of cotton batting on a shelf exposed to the light, and cover with a large inverted beaker. In 1 week weigh again.

QUESTIONS

1. Tabulate the weights before and after drying and also the loss or gain in weight.
2. What happens chemically when an oil dries?
3. How can you account for the spontaneous combustion of oily rags?

Experiment 44 *

Determination of the Acid Number

Obtain a sample of oil, to be used in this experiment and the two following ones, in a small Erlenmeyer flask. Fit the flask

with a two-hole rubber stopper, and through one of the holes pass a glass tube about 4 in. long to serve as a pipette for removing samples for analysis. Weight the flask with its oil, record the weight, transfer about 5 cc. to an Erlenmeyer flask, and weigh again. The loss in weight is the weight of the sample removed. Place 25 cc. of 95 per cent alcohol in a flask, add a drop of phenolphthalein, heat to boiling, and add 0.1 *N* KOH solution until a faint pink remains. Add this neutral alcohol to the sample of oil, heat to boiling, and titrate to a faint pink color with 0.1 *N* KOH solution.

QUESTIONS

1. What was the weight of the flask and oil before and after removing the sample, and what was the weight of the sample?
2. How many cubic centimeters of KOH did you use?
3. What was the normality of the KOH solution?
4. How many cubic centimeters of normal KOH solution would have been used?
5. How many milligrams of KOH are there in 1 cc. of normal KOH?
6. How many milligrams of KOH were used in the titration?
7. How many milligrams of KOH were used per gram of oil?
8. Define the acid number.
9. What is the significance of the acid number in an oil analysis?

Experiment 45 *

Determination of the Saponification Number

Transfer about 5 cc. of oil, accurately weighed as in Experiment 44, to a 250-cc. Erlenmeyer flask. Being very careful not to get the solution into your mouth, transfer by means of a pipette 50-cc. portions of 0.5 *N* alcoholic KOH solution[4] to the flask containing the sample of oil and to a similar flask containing no oil. This second flask is a blank. Fit each flask with a reflux air condenser, and heat on a steam bath for 30 minutes. Remove the reflux condensers, add 1 cc. of phenolphthalein indicator to

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[4] Place 28 grams of KOH in a 1000-cc. graduated flask. Add about 500 cc. of 95 per cent ethyl alcohol, and when the KOH is dissolved make up to volume with alcohol and mix thoroughly.

each flask, and titrate with 0.5 *N* HCl until the pink color just disappears.

QUESTIONS

1. Define the saponification number.
2. What is the significance of the saponification number in an oil analysis?
3. What was the weight of the sample flask before and after removing the sample, and what was the weight of the sample?
4. How many cubic centimeters of 0.5 *N* HCl were used for the sample and for the blank, and what is the difference between the two titrations?
5. What is the normality factor of the HCl?
6. How many cubic centimeters of normal HCl are equivalent to the difference of the above titrations?
7. To how many milligrams of KOH is this equivalent?
8. How many milligrams of KOH were required to saponify 1 gram of oil?
9. Define ester number.
10. From the results of this experiment and of Experiment 44 calculate the ester number of the oil.
11. What is the significance of the ester number in a fat analysis?

Experiment 46 *

Determination of the Iodine Number

Transfer to a 300-cc. glass-stoppered bottle about 0.3 gram of oil, accurately weighed, and add 10 cc. of chloroform. Add 25 cc. of Hanus's iodine solution[5] from a pipette, mix thoroughly to dissolve the oil, and allow to stand for 30 minutes. A blank determination should be run at the same time, using everything but the oil. Remove the stoppers from the bottles, and rinse them into their bottles with 10 cc. of 15 per cent KI solution.

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[5] Hanus's iodine solution: Place 13.2 grams of iodine in a large beaker, and add 1000 cc. of glacial acetic acid in small portions at a time while heating on a steam bath. When all the iodine has dissolved, cool, and titrate 5 cc. with standard sodium thiosulfate solution, using starch solution as an indicator. Now add bromine in sufficient quantity (about 3 cc.) to double the halogen content as indicated by titration. In the second titration after bromine has been added, 5 cc. of 15 per cent KI solution must be added before titrating with sodium thiosulfate so that all the free halogen will be in the form of iodine.

From a burette add 0.1 *N* sodium thiosulfate solution[6] until the solution becomes a light tan in color, indicating that most of the free iodine is gone. At this point add 5 cc. of starch solution,[7] and continue adding sodium thiosulfate solution until the last drop removes the blue color.

QUESTIONS

1. Define the iodine number.
2. What is the significance of the iodine number in an oil analysis?
3. What is the composition of Hanus's iodine solution?
4. Why is KI added before titrating with sodium thiosulfate?
5. How does $Na_2S_2O_3$ react with iodine? Write the equation.
6. Considering the saponification number and the iodine number of your oil, what oil do you think you have?
7. State the weight of your sample bottle of oil before and after removing the sample and also the weight of your sample.
8. State the number of cubic centimeters of sodium thiosulfate solution used for titrating the blank and the sample and the difference between the titrations.
9. To how many grams of iodine is 1 cc. of the sodium thiosulfate solution equivalent?
10. What is the weight of iodine absorbed by your sample of oil?
11. How many grams of iodine would be absorbed by 100 grams of oil?

Experiment 47

Preparation of Lecithin

Place about 10 grams of finely divided brain in an Erlenmeyer flask, and add 50 cc. of ethyl ether. Stopper the flask, and allow

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[6] Sodium thiosulfate solution: Prepare an approximately 0.1 *N* solution of sodium thiosulfate by dissolving 25 grams of sodium thiosulfate in 1000 cc. of water. Transfer 3.8633 grams of pure $K_2Cr_2O_7$ to a 1000-cc. volumetric flask, dissolve in a little water, and make up to volume with water. This solution is used for standardizing the sodium thiosulfate solution, and each cubic centimeter is equivalent to 0.01 gram of iodine. By means of a pipette transfer 20 cc. of the $K_2Cr_2O_7$ solution to an Erlenmeyer flask, and add 20 cc. of water, 10 cc. of 15 per cent KI solution, and 5 cc. of concentrated HCl. Titrate with the sodium thiosulfate solution until the solution is light tan in color, and then add 2 cc. of starch solution and titrate until the blue color changes to sea green. Express the strength of the sodium thiosulfate solution in terms of the grams of iodine equivalent to 1 cc., bearing in mind that 1 cc. of the $K_2Cr_2O_7$ solution is equivalent to 0.01 gram of iodine.

[7] Starch solution: Place 0.5 gram of starch in a test tube, and make a suspension in cold water. Heat 100 cc. of water in a beaker to boiling, and add the starch suspension slowly with constant stirring.

the mixture to stand until the next laboratory period. Filter, and add acetone to the filtrate until a precipitate is formed. Filter, transfer the precipitate to a crucible, mix with five times its bulk of fusion mixture[8] and ignite. Heat over a free flame until smoking ceases and the mixture melts. Cool, dissolve in water, acidify with HNO_3, heat to 65°C., and add a little ammonium molybdate solution.[9]

QUESTIONS

1. What is the composition of the fusion mixture?
2. What are the color and name of the final precipitate?
3. What has been proved to be present in lecithin?
4. What might happen if the solution were heated above 65°C.?
5. Write the formula for lecithin.

Experiment 48

Unsaponifiable Matter

Place about 3 grams of lard in an Erlenmeyer flask, add 25 cc. of 0.5 N alcoholic KOH solution, and heat for 1 hour on a steam bath under a reflux air condenser. Add 25 cc. of cold water, and transfer the mixture to a separatory funnel. Add 20 cc. of petroleum ether, shake, and transfer the ether layer to an evaporating dish. Repeat the ether extraction, transferring the ether layer to the evaporating dish. Evaporate the ether on a steam bath, and examine the residue in the evaporating dish.

QUESTIONS

1. What happened to the lard on boiling with alcoholic KOH?
2. What happened on adding water?
3. What are the main constituents of the unsaponifiable matter of plant and animal fats?
4. What is the importance of this unsaponifiable matter in vitamin studies?
5. Write the formula for cholesterol.

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[8] Fusion mixture: Mix 1 part of KNO_3 and 2 parts of anhydrous Na_2CO_3.

[9] Ammonium molybdate solution: Add 100 grams of molybdic acid to 144 cc. of ammonium hydroxide (sp. gr. 0.90) and 271 cc. of distilled water, and stir until the molybdic acid is dissolved. With constant stirring pour this solution slowly into a solution of 489 cc. of nitric acid (sp. gr. 1.42) in 1148 cc. of distilled water. Allow the solution to stand in a warm place for several days or until a portion heated to 40°C. gives no yellow precipitate. Decant the clear supernatant liquid for use.

Experiment 49

Cholesterol

1. Salkowski's Test. Place 3 cc. of a 1 per cent solution of cholesterol in chloroform in a test tube, and layer with concentrated H_2SO_4. Observe any color produced at the juncture of the two layers.

2. Liebermann-Burchard Test. Place 3 cc. of a chloroform solution of cholesterol in a dry test tube; add 10 drops of acetic anhydride and 3 drops of concentrated H_2SO_4. Mix, and notice the colors produced.

QUESTIONS

1. Describe the color changes in the tests.
2. Where is cholesterol found?

PROTEINS

Experiment 50

Composition of Proteins

1. Heat a small amount of dried egg albumin in a test tube, and notice the odor.

2. Thoroughly mix equal portions of powdered egg albumin and soda lime, and heat in a test tube. Test the fumes arising from the mouth of the test tube with moist red litmus paper.

3. Thoroughly mix in a mortar approximately 1-gram portions of iron filings, flaked sodium hydroxide, and powdered egg albumin. Transfer to a crucible, and heat with a low flame until the material is well charred and no more smoke comes off. Cool, add a few cubic centimeters of distilled water, and heat gently to dissolve as much of the material as possible. Filter, and acidify the filtrate with concentrated HCl. If nothing happens, add a drop of 10 per cent $FeCl_3$ solution.

4. Place about 2 grams of a mixture of equal parts of dried egg albumin and fusion mixture[1] in a crucible, and heat until the mixture is colorless. Cool, and dissolve in water, heating if necessary. Filter, acidify the filtrate with HCl, and add a few drops of 10 per cent $BaCl_2$ solution.

QUESTIONS

1a. Describe the odor.
  b. Is this odor characteristic of all burning protein?
2a. What is soda lime?
  b. What appeared on the sides of the tube?
  c. What happened to the litmus paper? Why?
  d. What element is shown to be present in protein by this test?
3a. What was the final color produced?
  b. What are the name and formula of the colored compound?
  c. What elements are shown to be present in protein by this test?
4a. What is the precipitate formed?
  b. What element does this test prove to be present in egg albumin?
  c. Name three amino acids in which this element is found.
  d. Why was it necessary to use fusion mixture in this test?

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[1] Fusion mixture: See page 110.

Experiment 51

Preparation of Edestin

Place 25 grams of ground hemp seed in an Erlenmeyer flask, and add 200 cc. of 5 per cent NaCl solution heated to 50°C. Immerse in a beaker of water kept at 50° to 55°C. for 1 hour, agitating frequently. A thermometer should be kept in the beaker of water to regulate its temperature. Filter, using a hot water funnel, through a filter paper which has been moistened with warm 5 per cent NaCl solution. Allow the filtrate to stand until cool, and then finally cool in cold water. When the edestin has settled out, decant the supernatant liquid, and filter and wash the precipitate with a few cubic centimeters of cold 5 per cent NaCl solution. Test the solubility of the edestin in water and 5 per cent NaCl solution heated to 50°C. Heat the solution in 5 per cent NaCl to boiling. Test some of the edestin to prove that it is protein. Save the bulk of the edestin for Experiment 52.

QUESTIONS

1. To what class of protein does edestin belong?
2. What would happen if the extraction were made above 55°C.?
3. Why should the filter paper be treated with hot 5 per cent NaCl solution before filtration?
4. Is edestin soluble in water? In 5 per cent NaCl solution?
5. What happened when the solution in 5 per cent NaCl was boiled?
6. How did you prove that edestin is a protein?

Experiment 52

Preparation of Edestan

Dissolve the edestin remaining from Experiment 51 in as little 0.2 per cent HCl as possible. Allow to stand for 30 minutes, and neutralize with 0.5 per cent Na_2CO_3 solution. Filter, and test the precipitate for its solubility in water, 5 per cent NaCl, 0.2 per cent HCl, and 0.5 per cent Na_2CO_3 solution. Also test the precipitate to prove that it is protein.

QUESTIONS

1. To what class of protein does edestan belong?
2. What happened when the edestin solution was neutralized with Na_2CO_3?

3. What is the solubility of edestan in water, 5 per cent NaCl, 0.2 per cent HCl, and 0.5 per cent Na_2CO_3?

4. How did you prove that edestan was a protein?

Experiment 53

Preparation of Gliadin and Glutenin from Wheat Flour

1. **Preparation of Gluten.** Make a stiff dough from about 50 grams of spring wheat flour. Knead thoroughly, and allow to stand for ½ hour to develop the gluten. Wash out all the starch by kneading in cold running water.

2. **Preparation of Gliadin.** Finely divide the gluten prepared, and place it in a flask containing 250 cc. of 70 per cent alcohol. Shake frequently, and allow it to stand until the next laboratory period. Filter, and evaporate the filtrate to dryness in an evaporating dish on a steam bath. Prove that the resulting material is protein.

3. **Preparation of Glutenin.** Transfer the material left on the filter in the preparation of gliadin to a flask, add 100 cc. of 70 per cent alcohol, and let stand until the next laboratory period. Separate the glutenin by filtration. Transfer the glutenin on the filter to a beaker, and add 100 cc. of 0.2 per cent NaOH solution. Allow to stand for 30 minutes, agitating frequently. Filter, and cautiously neutralize the filtrate with 0.2 per cent HCl solution until a maximum precipitation occurs. Filter; wash with 70 per cent alcohol and then with water. Test the solubility of the glutenin in water, 5 per cent NaCl, 0.2 per cent HCl, and 0.5 per cent Na_2CO_3. Prove that glutenin is a protein.

QUESTIONS

1a. Of what two proteins is gluten composed?

b. Of what importance is gluten in bread making?

2a. To what class of protein does gliadin belong?

b. Why must evaporation be done on a steam bath?

c. How did you prove gliadin to be a protein?

3a. To what class of protein does glutenin belong?

b. What is its solubility in water, 5 per cent NaCl, 0.2 per cent HCl, and 0.5 per cent Na_2CO_3?

c. How did you prove glutenin to be a protein?

Experiment 54

Preparation of Casein

To 100 cc. of milk in a beaker slowly add 10 per cent lactic acid solution, with constant stirring, until the casein precipitates. Filter, and wash the precipitate with a little acetone. Mix equal parts of the casein and fusion mixture[1] in a crucible, and ignite. Cool, dissolve in water, acidify with HNO_3, heat to 65°C., and add ammonium molybdate solution.[2]

QUESTIONS

1. What kind of protein is casein?
2. What is the isoelectric point of casein?
3. What is the acid in sour milk?
4. What happens to milk when it sours?
5. What is removed from the precipitate by washing with acetone?
6. What does the test with ammonium molybdate prove is present in casein?

Experiment 55 *

Preparation of Nucleoprotein

Place 25 grams of compressed yeast, 5 cc. of ethyl ether, and 15 cc. of water in a mortar, and grind thoroughly together with a little sand. Transfer the mixture to an Erlenmeyer flask, and add 100 cc. of 0.4 per cent NaOH solution, together with a few drops of toluene. Allow the mixture to stand until the next laboratory period, shaking occasionally. Filter, and add to the filtrate 5 per cent HCl solution, drop by drop, until maximum precipitation occurs. If too much HCl is added, the precipitate will dissolve; if this happens, add 1 per cent NaOH solution until maximum precipitation occurs. Filter, and test a small portion of the precipitate for protein and another small portion for phosphorus. The phosphorus test is made by mixing the nucleoprotein with twice its weight of fusion mixture[1] and igniting in a crucible. The fused material is dissolved in water, acidified with HNO_3, heated to 65°C., and treated with ammonium molybdate solution.[2]

Transfer the main bulk of the nucleoprotein to a beaker, and

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[2] **Ammonium molybdate solution: See page 110.**

boil for 1 hour with 50 cc. of 5 per cent H_2SO_4 solution, maintaining the original volume by frequent additions of water. Test one portion of this solution for purines by making faintly alkaline with NH_4OH, filtering if necessary, and to the clear solution adding ammoniacal silver nitrate solution.[3] Test the second portion of the solution for carbohydrate by means of Molisch's test.[4]

QUESTIONS

1. What was the purpose of grinding with sand?
2. What was the purpose of the toluene?
3. To what class of protein does nucleoprotein belong?
4. Indicate the chemical structure of nucleic acid.
5. In what types of material is nucleoprotein found in largest quantities?
6. How did you test for phosphorus in nucleoprotein?
7. What happened to the nucleoprotein on boiling with H_2SO_4?
8. How did you test for purines?
9. Name the purines found in nucleic acid.
10. What is the final oxidation product of these purines in the human body?
11. What is the carbohydrate present in yeast nucleic acid?
12. How did you test for carbohydrate?

Experiment 56

Preparation of Acid Metaprotein

Wash 25 grams of ground lean meat free from blood. Place in a beaker, together with 100 cc. of 0.2 per cent HCl. Heat on a steam bath for ½ hour, filter, cool, and add 0.2 per cent NaOH solution to the filtrate until maximum precipitation occurs. Filter, and prove that the precipitate is protein.

QUESTIONS

1. To what class of protein does acid metaprotein belong?
2. How did you prove the precipitate to be a protein?
3. What do you understand a primary derived protein to be?

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[3] Ammoniacal silver nitrate: Dissolve 13 grams of $AgNO_3$ in 250 cc. of water, and add NH_4OH until the precipitate which forms just dissolves. Make up to a volume of 500 cc. with water.

[4] Molisch's test: See page 56.

Experiment 57

Preparation of Alkali Metaprotein

Add to a concentrated solution of egg albumin 50 per cent NaOH solution, drop by drop, with constant stirring until a jelly results. Cut the jelly into small pieces, and soak in several changes of water to remove the NaOH. Finally dissolve the jelly in water by heating gently. Cool, and add dilute HCl until maximum precipitation occurs. Filter, and prove that the precipitate is a protein.

QUESTIONS

1. To what class of protein does alkali metaprotein belong?
2. How did you prove it to be a protein?

Experiment 58

Proteoses and Peptones

Place 50 cc. of a concentrated solution of proteose-peptone[5] in a beaker, and add an equal volume of saturated $(NH_4)_2SO_4$ solution. Heat to boiling, and saturate with solid $(NH_4)_2SO_4$. Transfer the sticky precipitate to a beaker, and dissolve in as little water as possible. Test for protein this solution and also the solution from which the sticky precipitate was removed.

QUESTIONS

1. To what class of protein do proteoses and peptones belong?
2. How can proteoses and peptones be made from egg albumin?
3. What do you understand a secondary derived protein to be?
4. What would be the easiest way to make one-half saturated solution of $(NH_4)_2SO_4$?
5. What happened on one-half saturation of the proteose-peptone solution with $(NH_4)_2SO_4$?
6. What happened on complete saturation?
7. What remained in solution?
8. How did you prove proteose and peptone to be protein?

Experiment 59

Color Reactions of Proteins

1. Biuret Test. a. Preparation of Biuret from Urea. Place a ¼-inch layer of urea in a dry test tube and heat carefully,

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[5] Witte's peptone contains proteose and serves well for this experiment.

continuing the heating until the melted material becomes solid. While heating, test the fumes given off with moist red litmus paper. Cool the residue, add 5 cc. of water, warm to dissolve, and filter. Make the biuret test on the filtrate as indicated in **b.**

b. Biuret Test. To 2 cc. of egg albumin solution in a test tube add 2 cc. of 10 per cent KOH solution, and mix; add 0.5 per cent $CuSO_4$ solution, drop by drop, with constant agitation, until a maximum color is produced.

2. Millon's Test. To 4 cc. of egg albumin solution in a test tube add a few drops of Millon's reagent,[6] and heat to boiling. Repeat the test on 2 per cent phenol solution, solid tyrosine, and solid gelatin.

3. Xanthoproteic Test. To 2 cc. of egg albumin solution in a test tube add 1 cc. of concentrated HNO_3. Heat to boiling, and continue heating until a clear solution results. Cool, and cautiously neutralize with 10 per cent NaOH solution. Repeat the test on a 2 per cent phenol solution and on a little solid salicylic acid.

4. Hopkins-Cole Test. Mix 2 cc. of egg albumin solution with an equal volume of Hopkins-Cole reagent[7] in a test tube, and layer with concentrated H_2SO_4. Observe the color at the juncture of the two liquids. Repeat the test on a solution of gelatin.

5. Liebermann's Test. To 0.5 gram of powdered egg albumin in a test tube add a few crystals of sucrose and 5 cc. of concentrated HCl. Heat to boiling, and note the color.

6. Acree-Rosenheim Test. To 2 cc. of egg albumin solution in a test tube add a few drops of formaldehyde solution (1:5000). Mix, and layer with concentrated H_2SO_4. Notice the color at the juncture of the two layers.

7. Ehrlich's *p*-Dimethylaminobenzaldehyde Test. To 1 cc. of egg albumin solution in a test tube add 1 cc. of concentrated

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[6] Millon's reagent: To 1 part by weight of mercury add 2 parts by weight of HNO_3 (sp. gr. 1.42). Warm until solution is complete, and add 2 volumes of distilled water. After several hours' standing decant the supernatant liquid, which is Millon's reagent.

[7] Hopkins-Cole reagent: Cover 10 grams of powdered magnesium in an Erlenmeyer flask with distilled water, and add slowly with constant mixing 250 cc. of cold saturated oxalic acid solution. The flask should be cooled under running water while adding the oxalic acid. Filter, acidify the filtrate with acetic acid, and dilute to a volume of 1 liter with distilled water.

HCL, and boil for ½ minute. Add 2 drops of a 5 per cent solution of *p*-dimethylaminobenzaldehyde in 10 per cent H_2SO_4 solution, and add a few drops of 0.5 per cent $NaNO_2$ solution.

8. Ehrlich's Diazo Test. To 1 cc. of a 0.5 per cent sulfanilic acid solution in 2 per cent HCl in a test tube add an equal volume of 0.5 per cent $NaNO_2$ solution. Mix, and in ½ minute add 1 cc. of egg albumin solution. Mix, and make alkaline with NH_4OH.

9. Reduced Sulfur Test. To a little powdered egg albumin in a test tube add about 10 cc. of 10 per cent NaOH solution, and boil for a few minutes. Add a few drops of lead acetate solution.

10. Ninhydrin Test. To 5 cc. of egg albumin solution in a test tube add a few drops of ninhydrin solution. Heat to boiling, and cool under running water.

11. Molisch's Test. To 5 cc. of egg albumin solution in a test tube add 1 drop of Molisch's reagent,[4] and layer with concentrated H_2SO_4.

QUESTIONS

1a. What happened to the litmus paper?

b. Write an equation indicating the reaction taking place in the preparation of biuret from urea.

c. What chemical structure in the protein molecule is responsible for the biuret test?

d. Of what value is the biuret test in studying the hydrolysis of proteins?

e. What color is produced in a positive biuret test?

2a. What is the composition of Millon's reagent?

b. What happened when Millon's reagent was added to egg albumin solution?

c. What happened on boiling?

d. What was the result of the tests with phenol, tyrosine, and gelatin?

e. Account for the result with gelatin.

f. Name the amino acid in protein responsible for a positive Millon's test and write its formula.

3a. What happened when HNO_3 was added to egg albumin solution?

b. What happened on boiling?

c. What was the color of the solution before and after neutralizing with NaOH?

d. Did phenol and salicylic acid respond to the test?

e. Write the formulas for phenol and salicylic acid.

f. Name the amino acids responsible for a positive xanthoproteic test in proteins and write their formulas.

4a. How is Hopkins-Cole reagent made?

b. What is the active constituent of this reagent?

c. What was the color at the juncture of the two liquids?

d. Name the amino acid responsible for this test and write its formula.

e. Is this amino acid present in gelatin?

5a. Describe the color changes.

b. Why is sucrose used in Liebermann's test?

c. What amino acid is responsible for this test?

6a. Describe the color changes.

b. What amino acid is responsible for the Acree-Rosenheim test?

c. How could you test for formaldehyde in milk?

7a. Write the formula for *p*-dimethylaminobenzaldehyde.

b. What is the reactive part of the *p*-dimethylaminobenzaldehyde molecule in Ehrlich's test?

c. Describe the color changes at the various stages of the test.

d. What amino acid is responsible for this test?

8a. Write the formula for sulfanilic acid.

b. What is formed when sulfanilic acid reacts with HNO_2 in HCl solution? Write the formula.

c. Name the two amino acids responsible for the diazo test and write their formulas.

d. Describe the color changes in this test.

9a. Name the amino acids responsible for the reduced sulfur test and write their formulas.

b. What happens when the protein is boiled with NaOH?

c. What happens when lead acetate is added?

d. Why does silverware turn black when used with eggs?

10a. What color was produced on cooling?

b. Write the formula for ninhydrin.

c. What structure in the protein molecule is responsible for the ninhydrin test?

11a. What color was produced at the juncture of the two layers?

b. What must have been present in egg albumin to make Molisch's test positive?

c. What class of protein would you expect to give a positive Molisch test?

Experiment 60

Precipitation of Proteins

1. Protein Salt. Make some egg albumin solution slightly acid to litmus by adding 5 per cent acetic acid solution. Test portions of this solution in test tubes by adding solutions of picric acid, potassium dichromate, potassium ferrocyanide, trichloracetic acid, and phosphotungstic acid.[8]

[8] Phosphotungstic acid: Dissolve 100 grams of sodium tungstate and 70 grams of disodium phosphate in 500 cc. of distilled water, and acidify with HNO_3.

2. Proteinate. Test portions of neutral egg albumin solution in test tubes with solutions of lead acetate, mercuric chloride, copper sulfate, calcium chloride, and magnesium sulfate.

3. Dehydration. Place 5 cc. of egg albumin solution in each of 2 test tubes. To one add solid $(NH_4)_2SO_4$ sufficient to saturate the solution, and to the other add 5 cc. of 95 per cent alcohol.

4. Coagulation by Heat. Slightly acidify 5 cc. of egg albumin solution in a test tube with 5 per cent acetic acid, and heat to boiling.

QUESTIONS

1a. What happened in each test?
  b. What is the name of the precipitate when picric acid was added?
2a. What happened in each test?
  b. What is the name of the precipitate when lead acetate was added?
3a. What happened in each test?
  b. What type of colloidal solution does albumin form?
  c. Why did the albumin precipitate in these tests?
4. What happened on heating?

Experiment 61

Conditions for Heat Coagulation

Place 5 cc. of neutral egg albumin solution in each of 4 test tubes. To one add 2 drops of 5 per cent acetic acid, to another 2 drops of 1 per cent Na_2CO_3 solution, and to a third 2 drops of 5 per cent NaCl solution. Do not add anything to the fourth tube. Mark the tubes, and place them in a small beaker of water with a thermometer in one of the tubes. Put a rubber stopper in a large beaker half filled with water, and place the small beaker containing the test tubes in the large beaker, resting the small beaker on the rubber stopper. Slowly heat the large beaker, and, assuming the temperature to be the same in all the tubes, note the temperature at which coagulation occurs in each tube.

QUESTIONS

1. At what temperature did coagulation start in each tube?
2. Does albumin coagulate more easily in acid or alkaline solution?

Experiment 62

Ring Tests for Protein

1. Heller's Ring Test. Place 5 cc. of egg albumin solution in a test tube, and layer with concentrated HNO_3.

2. Roberts's Ring Test. Place 5 cc. of egg albumin solution in a test tube and layer with Roberts's reagent.[9]

QUESTIONS

1. What happened in each test?
2. What is the composition of Robert's reagent?
3. How may the precipitation of protein by acid be explained?

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[9] Roberts's reagent: To 5 volumes of saturated $MgSO_4$ solution add 1 volume of concentrated HNO_3.

FOODS

Experiment 63 *

Determination of Total Solids in Milk

Thoroughly mix a sample of milk by pouring it several times from one vessel to another. By means of a lactometer (see Fig. 14) determine its specific gravity. Heat a flat-bottom porcelain dish in a free flame, cool in a desiccator for 30 minutes, and weigh. Add to the weighed dish 5 cc. of milk from a pipette, and evaporate to dryness on a steam bath. Finally dry in a 100°C. oven for 2½ hours, cool in a desiccator for 30 minutes, and weigh. The increase in weight of the dish is the weight of the total solids in the milk. The weight of the sample of milk in grams is 5 times the specific gravity of the milk. The weight of the total solids divided by the weight of the sample times 100 gives the percentage of total solids in the sample.

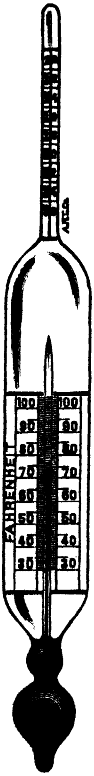

FIG. 14. Lactometer.

QUESTIONS

1. What is the specific gravity of the milk?
2. What is the weight of 5 cc.?
3. What is the weight of the porcelain dish?
4. What is the weight of the porcelain dish plus the total solids?
5. What is the weight of the total solids?
6. What is the percentage of total solids in the milk?
7. What is the percentage of water in the milk?
8. Why should the dish be cooled in a desiccator before weighing?

Experiment 64 *

Determination of Ash in Milk

Place the porcelain dish containing the total solids from Experiment 63 on a clay triangle, and heat slowly over a free flame until all the volatile matter is gone. Do not allow the solids to catch on fire. Finally heat to

a low red heat until the ash is white or light gray in color. Cool in a desiccator for 30 minutes, and weigh.

QUESTIONS

1. What is the weight of the dish plus the ash?
2. What is the weight of the dish?
3. What is the weight of the ash?
4. What is the weight of the original sample of milk?
5. What is the percentage of ash in the milk?
6. Why not heat to a white heat?

Experiment 65 *

Determination of Fat in Milk by the Adams Paper Coil Method

Pin a strip of special fat-free paper[1] to the edge of your laboratory desk and deliver from a pipette 5 cc. of a thoroughly mixed sample of milk, whose specific gravity is known, onto the center of the strip, being careful not to allow the milk to soak to the edge of the strip. Allow the strip to dry in the air; then roll into a coil, and bind with a copper wire. Place the coil in a small beaker, and dry in an oven at 100°C. for 2 hours. Place the coil in an extractor (see Fig. 15), attach to a condenser, and attach to the bottom of the extractor a small weighed flask. Use cork stoppers to attach the flask to the extractor and the extractor to the condenser, and make sure that the stoppers are tight. Place 50 cc. of anhydrous ethyl ether in the extractor, and extract for 2 hours, heating the extraction flask in a water bath kept at a temperature which will cause the ether to drip from the condenser at a fairly rapid rate. Take the flask from the extractor, and remove the ether by heating on a steam bath. Finally dry the flask by heating in an oven at 100°C. for 2½ hours. Cool in a desiccator for 30 minutes, and weigh.

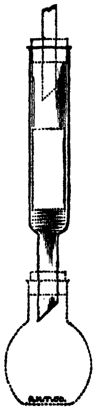

FIG. 15. Extraction apparatus.

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[1] Fat-free paper: Strips of fat-free paper for use in this determination may be obtained from any chemical supply house.

QUESTIONS

1. Why must the sample be dry before extraction?
2. Why must anhydrous ethyl ether be used?
3. What is the specific gravity of the milk?
4. What is the weight of 5 cc. of milk?
5. What is the weight of the extraction flask plus the fat?
6. What is the weight of the flask?
7. What is the weight of the fat?
8. What is the percentage of fat in the milk?
9. What percentage of fat must be present in milk in order that it may be sold as market milk?

Experiment 66 *

Colorimetric Method for the Determination of Lactose in Milk[2]

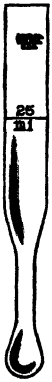

Fig. 16. Folin-Wu sugar tube.

Place exactly 1 cc. of well-mixed milk, the specific gravity of which is known, in a 100-cc. graduated flask, and add 2 cc. of 10 per cent sodium tungstate solution. Add, drop by drop, 2 cc. of 0.66 N H_2SO_4 solution with constant shaking, and allow to stand for 5 minutes. Dilute to the mark with distilled water, mix well, and filter. Place 1 cc. of the filtrate and 1 cc. of water into a Folin-Wu sugar tube. (See Fig. 16.) In a similar tube place 2 cc. of standard lactose solution[3] containing 0.6 mg. of lactose. Add to each tube 2 cc. of Folin-Wu alkaline copper reagent,[4] and place the tubes in a beaker of boiling water for 8 minutes.

Cool, and add 2 cc. of Folin-Wu phosphomolybdic acid solution.[5] Allow to stand for 1

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[2] Method of Owen and Gregg, modified.

[3] Standard lactose solution: Dissolve 1 gram of lactose in a saturated solution of benzoic acid, and make up to a volume of 100 cc. with saturated benzoic acid solution. Place 3 cc. of this solution in a 100-cc. volumetric flask and make up to volume with saturated benzoic acid solution. This solution contains 0.6 mg. of lactose in 2 cc.

[4] Alkaline copper reagent: Dissolve 40 grams of anhydrous Na_2CO_3 in 400 cc. of water in a 1000-cc. volumetric flask. Add to this solution 7.5 grams of tartaric acid. When this has dissolved, add 4.5 grams of crystallized copper sulfate. When this has dissolved, make up to volume with water and mix. If a precipitate appears in this solution on standing, it should be filtered.

minute; then dilute to the 25-cc. mark with water. Mix thoroughly, and compare colors in a colorimeter, setting the standard of 20 mm. In calculating results bear in mind that the intensity of the color is proportional to the amount of lactose present, and also that the depth to which the plunger is set in the unknown is inversely proportional to the intensity of the color. Use the following formula for calculation:

$$\frac{\text{Reading of standard} \times 0.6}{\text{Reading of unknown} \times 0.01} = \text{Milligrams of lactose in 1 cc. of milk}$$

Taking into account the specific gravity of the milk, calculate the percentage of lactose present.

QUESTIONS

1. What is formed when sodium tungstate and H_2SO_4 are mixed?
2. What does this compound do to the milk?
3. What is the composition of the alkaline copper reagent?
4. What is the precipitate in the Folin-Wu sugar tube after heating?
5. Why is the Folin-Wu sugar tube constricted?
6. What is the composition of the acid molybdate solution?
7. What color is produced when this solution is added to the Folin-Wu tubes?
8. Explain the production of the color.
9. Calculate the milligrams of lactose in 1 cc. of milk, using the formula given in this experiment.
10. What is the specific gravity of the milk?
11. What is the percentage of lactose in the milk?

Experiment 67 *

Determination of Protein in Milk by a Modified Kjeldahl Method

Place 5 cc. of a well-mixed sample of milk, the specific gravity of which is known, in a Kjeldahl flask, and add 10 grams of Na_2SO_4, a small crystal of $CuSO_4$, and 25 cc. of concentrated H_2SO_4. To another flask, which will serve as a blank, add everything but the milk. Place the flasks on a digestion rack

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[5] Phosphomolybdic acid solution: Place 35 grams of molybdic acid and 5 grams of sodium tungstate in a large beaker, add 200 cc. of 10 per cent NaOH solution and 200 cc. of water, and boil for 30 minutes to remove any ammonia which may be present. Cool, dilute to about 350 cc., and add 125 cc. of 85 per cent phosphoric acid. Transfer to a 500-cc. volumetric flask, and make up to volume with water.

(see Fig. 17), and digest until the solution in the flask containing the sample is clear.

Cool, cautiously add 250 cc. of distilled water, and mix thoroughly. Mark two Erlenmeyer flasks to correspond with the Kjeldahl flasks, and add to each 25 cc. of a saturated boric acid solution. The boric acid solution may be measured roughly in a graduate. Add a few drops of mixed indicator[6] to each flask. Place the flasks under the distillation rack (see Fig. 18) so that the tips of the delivery tubes nearly touch the liquid in the flasks. It is important that the condensers be clean and free from alkali. To insure clean condensers distil about 50 cc. of water through them from Kjeldahl flasks just before using. Add to each Kjeldahl flask a few drops of phenolphthalein, and cautiously pour down the side of the neck of the flask 60 cc. of saturated NaOH solution, being careful not to allow the alkali to touch the mouth of the flask. Alkali on the mouth of the flask will prevent the stopper from sticking properly. The alkali should run down the side of the neck of the flask gently and should form a layer in the bottom of the flask. Do not mix until the flask is connected to the distillation apparatus. Just before connecting the flask, add a few pieces of mossy zinc to prevent bumping. Connect the flask at once, and mix the contents thoroughly. If sufficient alkali has been added, the solution should be red. The

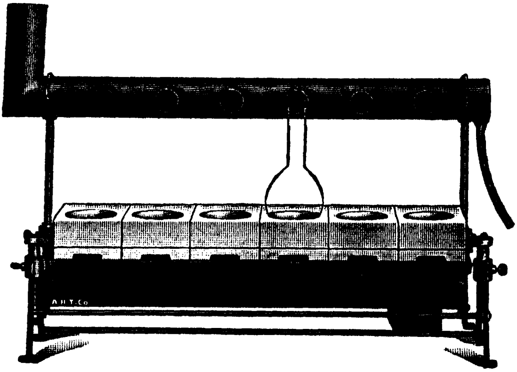

FIG. 17. Kjeldahl digestion apparatus.

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[6] Mixed indicator: Dissolve 1.25 grams of methyl red and 0.825 gram of methylene blue in 1 liter of 90 per cent ethyl alcohol.

color may fade because of a reduction of the phenolphthalein by the hydrogen produced by the action of the NaOH on the zinc. Light the burner, and heat with a small flame. Adjust the Erlenmeyer flasks so that the delivery tubes dip beneath the surface of the acid. When the liquid in the Kjeldahl flasks boils and you have satisfied yourself that there is no danger of boiling over, increase the flames and distil about 175 cc. into the receiving flasks. If at any time you need to remove the flame from a flask, move the receiving flask so that the delivery tube is not dipping in the distillate. This is done to prevent the distillate from being sucked back into the Kjeldahl flask. When distillation is complete, disconnect the delivery tubes, and rinse them into their receiving flasks with distilled water. Return the delivery tubes to the distillation apparatus. At the time the receiving flasks are removed, turn off the flames. To preserve the elasticity of the stoppers, loosen them in the Kjeldahl flasks before they cool.

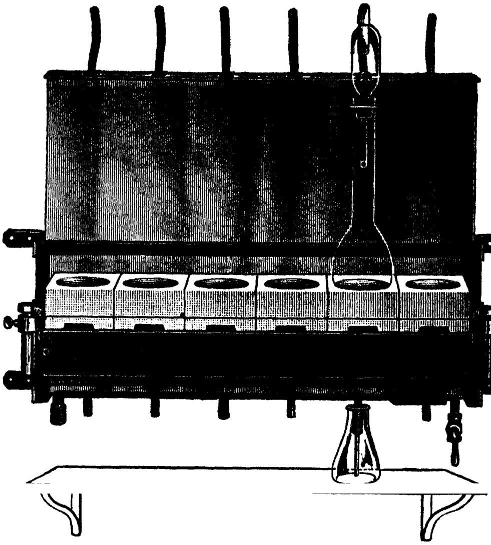

FIG. 18. Kjeldahl distillation apparatus.

The receiving flasks now contain all the ammonia distilled from the Kjeldahl flasks. The ammonia is held in solution as ammonium borate. The solution is basic and should have a green color due to the indicator. Since boric acid is a very weak

acid, it reacts as a base to the mixed indicator used. Therefore the ammonia present may be titrated directly with standard acid. Titrate the sample and the blank with 0.1 *N* HCl solution. The end point is a purple color. The difference in titration of the blank and the sample gives the number of cubic centimeters of HCl solution equivalent to the NH_3 distilled. This difference times the normality factor of the HCl solution times 0.014 gives the grams of nitrogen in the sample. To convert this nitrogen into protein, multiply by the factor 6.38. Taking into account the specific gravity of the milk, calculate the percentage of protein in the milk. The following formula may be used in your calculation:

$$\frac{\begin{bmatrix}\text{cc. HCl} - \text{cc. HCl}\\ \text{(sample)} \quad \text{(blank)}\end{bmatrix} \times \text{N.F.} \times 0.014 \times 6.38 \times 100}{5 \times \text{Specific gravity of sample}} = \%\ \text{protein}$$

QUESTIONS

1. State the functions of the H_2SO_4, Na_2SO_4, $CuSO_4$, Zn, and saturated NaOH solution in this experiment.
2. Why is a blank determination necessary?
3. In what form is the nitrogen after digestion?
4. What happens to the nitrogen when the solution is made alkaline?
5. Does the method of this experiment include nitrate nitrogen?
6. What is the specific gravity of the milk?
7. What is the weight of 5 cc. of milk?
8. What is the normality of the standard HCl solution used?
9. How many cubic centimeters of HCl solution are used for the blank? For the sample? What is the difference?
10. To how many cubic centimeters of normal HCl is this difference equal?
11. To what weight of nitrogen is this equal?
12. What is the factor used for converting nitrogen into protein?
13. Why is the factor of 6.25 not used?
14. What is the weight of protein in 5 cc. of milk?
15. What is the percentage of protein in the milk?
16. Summarize the composition of your sample of milk for total solids, water, ash, fat, lactose, and protein.
17. What percentage of the total solids do the ash, fat, lactose, and protein account for?

Experiment 68

Separation of the Important Constituents of Milk

1. Casein. To 100 cc. of milk in a beaker add, with stirring, 10 per cent lactic acid solution until the casein precipitates. Filter,

and save the filtrate for the subsequent experiments. Transfer the precipitate to a beaker, and add 25 cc. of acetone. Shake well, and decant the acetone through a dry filter catching the fitrate in an evaporating dish. Repeat the extraction with acetone, this time transferring the casein to the filter. Dry the casein, and test it to prove that it is protein.

2. Fat. Evaporate the acetone solution in the evaporating dish to dryness on a steam bath, and observe the residue.

3. Lactose. Test some of the filtrate saved from the filtration of casein with Fehling's solution.

4. Globulin. To the remainder of the filtrate from the casein add a few drops of phenolphthalein indicator, and then add milk of lime until a faint pink color appears. The precipitate formed is composed largely of phosphates. Filter, and make the filtrate neutral to litmus with 1 per cent HCl solution. To this solution add an equal volume of saturated $(NH_4)_2SO_4$ solution. Filter, and test the precipitate to prove that it is protein.

5. Albumin. To the filtrate from **4** add 5 per cent H_2SO_4 solution, drop by drop with stirring, until maximum precipitation occurs. The lactalbumin precipitates best at its isoelectric point, which is at a *p*H of about 4.7. Filter, and test the precipitate to prove that it is protein.

QUESTIONS

1a. At what *p*H does casein precipitate best?
  b. Why did you extract with acetone?
  c. To what class of protein does casein belong?
  d. How did you prove casein to be a protein?
2a. What was the residue after evaporating the acetone?
  b. What were its color and odor?
3a. What was the result of the Fehling's test?
  b. What substance in milk was responsible for this test?
  c. What does lactose give on hydrolysis?
4a. What happened on adding milk of lime?
  b. What was precipitated at this point?
  c. Is a globulin soluble in water?
  d. What precipitated on one-half saturation with $(NH_4)_2SO_4$?
5a. What is the isoelectric point of lactalbumin?
  b. What was the purpose of adding H_2SO_4?

ENZYMES

Experiment 69

Preparation of Sucrase

Mix 50 grams of compressed yeast with about 10 grams of sand, and grind thoroughly in a mortar. Spread the mixture on a large filter paper, and dry in a hot air oven at a temperature not to exceed 60°C. When the mixture is dry, grind it in a mortar again, and transfer to a beaker containing 50 cc. of water. Mix thoroughly, allow to stand for 10 minutes, and filter through a coarse filter, collecting the filtrate in a beaker containing 50 cc. of acetone. When filtration is complete, mix the filtrate and acetone well, allow to stand for a few minutes, and filter. Dry the precipitate in a hot air oven kept at 60°C. or less. Save this sucrase for the next experiment.

QUESTIONS

1. Why is the yeast ground with sand?
2. Why should the mixture not be heated above 60°C.?
3. What is the precipitate in the acetone?

Experiment 70

Demonstration of Typical Enzymes

1. Sucrase. Place 5 cc. of 1 per cent sucrose solution in each of 2 test tubes. Heat 1 tube to boiling, and while it is still boiling hot add to each tube a pinch of the powdered sucrase prepared in Experiment 69. Continue boiling the heated tube for 1 minute. Cool, and place both tubes in a beaker of water kept at 38°C. for 2 hours. Test both solutions with Fehling's solution.

2. Amylase. Place 5 cc. of starch solution in each of 2 test tubes. Heat 1 tube to boiling, add 1 cc. of saliva, continue the boiling for 1 minute, and cool. Next add 1 cc. of saliva to the unboiled tube. Place both tubes in a beaker of water at 38°C.,

and heat at this temperature for 30 minutes. At the end of this time test both solutions with Fehling's solution and with iodine solution.[1]

3. Lipase. Place 5 cc. of litmus milk[2] in each of 2 test tubes. Heat 1 tube to boiling, add a pinch of pancreatin as a source of lipase, and continue heating for 1 minute. Cool, and add a pinch of pancreatin to the other tube. Place in a water bath at 38°C. for 2 hours. Observe the color of the litmus in both tubes.

4. Protease. Into each of 2 test tubes put 5 cc. of a 1 per cent solution of pepsin dissolved in 0.2 per cent HCl solution. Heat 1 tube to boiling, and add a piece of fibrin. Continue the boiling for 1 minute; then cool. Next add a piece of fibrin to the other tube. Place both tubes in a beaker of water at 38°C. for 2 hours. Note whether there is any evidence of the fibrin dissolving in either tube.

5. Coagulase. Place 5 cc. of milk in each of 2 test tubes. To 1 tube add 10 drops of saturated ammonium oxalate solution, and mix. Add to both tubes 5 drops of 1 per cent rennin solution, and place both tubes in a beaker of water kept at 38°C. for 15 minutes. Observe what happens in each tube.

6. Catalase. Pare a small potato, and grate it to a fine pulp. Mix this pulp with 100 cc. of water, let stand for 15 minutes, and strain through a piece of cheesecloth. Filter the extract. Divide the filtrate into two parts, and boil one part for 1 minute. Place 5 cc. of the boiled filtrate in one tube and 5 cc. of the unboiled filtrate in another, and add to each a few drops of hydrogen peroxide. Note what happens. Next add to each tube 1 cc. of a 1.5 per cent solution of guaiac resin in 95 per cent alcohol. Note any color change.

QUESTIONS

1a. What happened when Fehling's test was applied to the solution in each tube?

b. What does a boiling temperature do to sucrase?

c. What happens to sucrose when it is acted upon by sucrase?

2a. What is the name of the starch-splitting enzyme in saliva?

b. What are vegetable starch-splitting enzymes called?

c. What happened in the Fehling's tests?

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[1] **Iodine solution:** See page 54.

[2] **Litmus milk:** Add 1 gram of powdered litmus to 500 cc. of milk, and mix thoroughly.

d. What happened in the iodine tests?
e. What does starch give on hydrolysis by ptyalin?
f. What does starch give on complete hydrolysis?
3a. What is pancreatin?
b. What enzymes would you expect it to contain?
c. What is the name of the pancreatic lipase?
d. What does pancreatic lipase do to fat?
e. What color change did you note in the 2 tubes? Explain.
f. What is the reaction of milk to litmus?
4a. Where is pepsin found?
b. At what *p*H does pepsin work best?
c. What happened in each tube?
d. What is fibrin?
e. What are the products of the action of pepsin on protein?
f. What is pepsinogen?
g. How is pepsinogen activated?
5a. Where is rennin found?
b. What does rennin do to casein?
c. What is the clot formed when rennin acts on casein?
d. What happened in each tube?
e. How does ammonium oxalate prevent the rennin clotting of milk?
6a. What does catalase do?
b. What happened in each tube after H_2O_2 was added?
c. How do you account for the color change in the tube where active catalase is present?

SALIVARY DIGESTION

Experiment 71

Reaction of Saliva

The saliva for this experiment and subsequent ones is obtained by chewing paraffin wax, which stimulates salivary secretion. Filter the saliva as it is obtained, and use the clear filtrate.

Test a few drops of saliva in a test tube with phenolphthalein, litmus, and Congo red, and from the colors produced estimate the approximate *p*H of your saliva. (See Table 1.)

QUESTIONS

1. At what *p*H values do phenolphthalein, litmus, and Congo red change color?
2. What is the color of these indicators in acidic and basic solution?
3. What color does each indicator give with your saliva?
4. What is the approximate *p*H of your saliva?
5. What is the *p*H of normal saliva?

Experiment 72

Composition of Saliva

1. **Mucin.** Acidify 2 cc. of saliva in a test tube with a few drops of a 5 per cent acetic acid solution.

2. **Protein.** Make a biuret test on 1 cc. of saliva. (See page 126.)

3. **Carbohydrate.** Make a Molisch test on 2 cc. of saliva. (See page 56.)

4. **Chloride.** Acidify 1 cc. of saliva with a drop of dilute HNO_3, and add a drop of $AgNO_3$ solution.

5. **Sulfate.** Acidify 1 cc. of saliva with a drop of dilute HCl, and add a drop of $BaCl_2$ solution.

6. **Phosphates.** Acidify 1 cc. of saliva with a drop of dilute HNO_3, add an equal volume of ammonium molybdate solution,[1] and heat to 65°C.

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[1] Ammonium molybdate solution: See page 110.

7. Calcium. Acidify 1 cc. of saliva with acetic acid, and add a drop of saturated ammonium oxalate solution.

8. Nitrites. Acidify 1 cc. of saliva with a drop of dilute H_2SO_4; add a few drops of KI solution and a drop of starch solution.[2]

9. Thiocyanates. Place a few drops of saliva in a crucible, add a few drops of $FeCl_3$ solution, and acidify with a drop of dilute HCl.

QUESTIONS

1a. What happened upon the addition of acetic acid?
  b. What was precipitated?
  c. What kind of a protein is mucin?
2a. What was the result of the biuret test?
  b. What is the main protein in saliva?
3a. What was the result of the Molisch test?
  b. Where is the carbohydrate found in saliva?
4a. What happened when $AgNO_3$ was added?
  b. Write an equation representing what happened.
5a. What happened when $BaCl_2$ was added?
  b. Write an equation representing what happened.
6. What happened when ammonium molybdate was added?
7a. What happened when ammonium oxalate was added?
  b. Write an equation representing what happened.
8. What happened in the test for nitrites?
9a. What happened in the test for thiocyanates?
  b. What is the formula for the colored compound formed?

Experiment 73

Preparation of Mucin

To 20 cc. of saliva in a beaker add 80 cc. of 95 per cent alcohol. Allow the precipitate to settle, pour off the supernatant liquid, and filter the precipitate from the remainder of the liquid. Dissolve the precipitate in as little water as possible, and test the solution for protein by the biuret test (see page 126) and for carbohydrate by the Molisch test (see page 56).

QUESTIONS

1. To what class of protein does mucin belong?
2. What is the function of mucin in the saliva?
3. What do the biuret and Molisch tests prove concerning the composition of mucin?

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[2] Starch solution: See page 108.

Experiment 74

Digestion of Starch by Ptyalin

Place 25 cc. of 1 per cent starch solution[2] in one beaker and 25 cc. of 1 per cent starch suspension in another. Warm to 38°C., and add to each beaker 1 cc. of saliva.

Prepare a spot plate with drops of iodine solution[3] in each depression. At intervals of 1 minute test drops of the contents of each beaker for starch on the spot plate. Stir the starch suspension thoroughly before removing the drops. After 10 minutes, tests may be made every 5 minutes. Determine the time required to reach the achromatic point in both solutions. When the achromatic point has been reached, test the solutions with Fehling's solution.

QUESTIONS

1. What does ptyalin do to starch?
2. What sugar is the final hydrolytic product when ptyalin acts on starch?
3. How long did it take to reach the achromatic point in each solution?
4. How do you account for the difference in time required to reach the achromatic point?
5. What was the result with Fehling's test? Explain.

Experiment 75

Influence of Conditions on Ptyalin Activity

1. Temperature. Prepare 4 test tubes, placing 5 cc. of starch solution[2] in each. Place the first tube in ice water, the second in a test-tube rack exposed to room temperature, the third in a beaker of water at 38°C., and the fourth in a beaker of boiling water. Add to each 2 drops of saliva, and mix. At frequent intervals test a drop of each solution with iodine solution[3] on a spot plate. Continue the testing for 1 hour. Notice the order in which the starch solutions reach the achromatic point.

2. Reaction. Place in a series of test tubes 5 cc. of buffer solutions[4] ranging in *p*H from about 3.0 to 9.0, the interval between solutions being 1 *p*H. Add to each tube 1 cc. of 1 per cent starch solution, and warm to 38°C. Dilute 1 cc. of saliva with 100 cc. of water, and add to each tube 1 cc. of the diluted

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[3] **Iodine solution:** See page 54.

[4] **Buffer solutions:** See page 40.

saliva. Mix thoroughly, and place the tubes in a beaker of water at 38°C. By means of the iodine test determine the time required for the achromatic point to be reached in each test tube. Continue the tests for 1 hour.

QUESTIONS

1a. What was the time required to reach the achromatic point in each test tube?

b. What do you consider the optimum temperature for the action of ptyalin?

2a. What was the time required to reach the achromatic point in each test tube?

b. What do you consider the optimum *p*H for ptyalin activity?

GASTRIC DIGESTION

Experiment 76

Products of Peptic Digestion

To 100 cc. of a concentrated solution of egg albumin add 0.4 per cent HCl solution until the mixture gives a blue color with Congo red indicator[1] when tested on a spot plate. Add 10 cc. of 1 per cent pepsin in 0.2 per cent HCl solution, and allow the mixture to stand until the next laboratory period, preferably in an incubator at 38°C. The solution should be tested occasionally with Congo red, and more HCl should be added if a blue color is not obtained. At the end of the digestion period filter, if any insoluble material is present, and neutralize the filtrate with 5 per cent NaOH solution. Any acid metaprotein should precipitate at this point. Filter, and saturate the filtrate with solid $(NH_4)_2SO_4$.

QUESTIONS

1. At what *p*H does Congo red turn color?
2. What is the *p*H of the solution if it just turns Congo red blue?
3. At what *p*H does pepsin act best?
4. What are the products of peptic digestion?
5. What is the precipitate on neutralization with NaOH?
6. What precipitates in saturated $(NH_4)_2SO_4$ solution?
7. What remains in solution?
8. What is the difference between a primary and a secondary derived protein?

Experiment 77

Effect of Reaction on Peptic Activity

Prepare 5 tubes, placing in the first 5 cc. of water, in the second 5 cc. of dilute HCl (desk reagent), in the third 5 cc. of 0.2 per cent HCl solution, in the fourth 5 cc. of 0.5 per cent Na_2CO_3, and in the fifth 5 cc. of 1 per cent Na_2CO_3 solution. Add to

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[1] Congo red: A 0.5 per cent alcoholic solution.

each tube a small piece of fibrin and 1 cc. of a 1 per cent pepsin solution. Place in a beaker of water at 38°C. for 2 hours, and observe any evidences of the fibrin having been acted upon by the pepsin.

QUESTIONS

1. What happened to the fibrin in each tube?
2. In which solution did the fibrin dissolve most rapidly?
3. At what *p*H does pepsin work best?

Experiment 78

Gastric Rennin

Place 5 cc. of milk in each of 5 test tubes. To the first tube add 0.1 *N* HCl solution, drop by drop, until the casein coagulates. To the second tube add 5 drops of 0.1 *N* HCl solution, to the third tube 10 drops of 5 per cent Na_2CO_3 solution, and to the fourth tube 10 drops of saturated ammonium oxalate solution. Next add 1 cc. of 1 per cent rennin solution to each tube except the first, and place these four tubes in a beaker of water at 38°C. Observe the time required for coagulation in each tube where it occurs. Continue the heating for 30 minutes. Finally place the tube containing ammonium oxalate in a beaker of boiling water for 5 minutes, cool, and add 10 per cent $CaCl_2$ solution slowly, drop by drop, until coagulation occurs.

QUESTIONS

1. What was precipitated in the first tube?
2. At what *p*H does casein precipitate?
3. How does the precipitate in the first tube compare chemically with that in the last tube?
4. What happened in tubes 2, 3, 4, and 5?
5. How do you explain the action in the tube containing ammonium oxalate?
6. What happened in this tube upon the addition of $CaCl_2$? Explain.
7. Was rennin necessary for coagulation after $CaCl_2$ was added?
8. What happened to the rennin on heating to boiling?
9. Does rennin work best in a neutral, acid, or alkaline medium?

Experiment 79*

Gastric Analysis

1. Total Acidity. Place 10 cc. of gastric contents[2] in a porcelain evaporating dish, and add 3 drops of phenolphthalein indicator. From a burette add 0.1 *N* NaOH solution until a faint pink color persists for 2 minutes. Calculate the number of cubic centimeters of 0.1 *N* NaOH necessary to neutralize 100 cc. of gastric contents.

2. Free Hydrochloric Acid. Place 10 cc. of gastric contents in a porcelain evaporating dish, and add 10 cc. of Sahli's reagent.[3] Mix well, and allow to stand for 5 minutes. Titrate with 0.1 *N* sodium thiosulfate solution[4] until a faint tan color remains. Add 1 cc. of 1 per cent starch solution,[5] and continue the titration with sodium thiosulfate solution until the blue color disappears. Calculate the number of cubic centimeters of 0.1 *N* sodium thiosulfate solution required for 100 cc. of juice. In this titration 1 cc. of 0.1 *N* $Na_2S_2O_3$ is equivalent to 1 cc. of 0.1 *N* HCl in the gastric contents. Each molecule of HCl liberates 1 atom of iodine from Sahli's reagent. The iodine is titrated with $Na_2S_2O_3$. The results of this titration are approximately the same as would be obtained if the stomach contents were titrated with 0.1 *N* NaOH solution, using as an indicator Töpfer's reagent, which changes color when free HCl is neutralized.

3. Lactic Acid (Strauss's Test). Place 5 cc. of stomach contents in a large test tube, add 20 cc. of ethyl ether, stopper, and

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[2] Gastric contents: Artificial gastric contents may be made by dissolving 5 grams of powdered egg albumin in 500 cc. of a 0.2 per cent solution of HCl and adding 5 cc. of lactic acid and a few drops of blood.

[3] Sahli's reagent: Mix equal volumes of 48 per cent KI and 8 per cent KIO_3 solutions. This solution should be colorless. On standing, it may turn brown because of the liberation of free iodine, which should be removed by adding 0.1 *N* $Na_2S_2O_3$ solution, drop by drop, until the brown color disappears.

[4] Sodium thiosulfate solution: Prepare 0.1 *N* $Na_2S_2O_3$ solution as in Experiment 46. Standardize it by titration against 0.1 *N* $K_2Cr_2O_7$ solution made by dissolving 4.9035 grams of pure $K_2Cr_2O_7$ in water and making up to 1000 cc. Follow the same procedure given in Experiment 46. Calculate the normality factor of the $Na_2S_2O_3$ solution.

[5] Starch solution: See page 108.

shake thoroughly. Remove the stopper; when the liquid has separated into two layers, transfer 5 cc. of the ether layer to another test tube. Add 20 cc. of water and 2 drops of 10 per cent $FeCl_3$ solution. Note the color produced.

4. Blood. Place 2 cc. of gastric contents in a test tube, and add 3 cc. of a fresh saturated solution of benzidine in glacial acetic acid. Add 1 cc. of 3 per cent hydrogen peroxide, and note the color.

QUESTIONS

1a. How are gastric acidities expressed?

b. How many cubic centimeters of NaOH solution were used in this titration?

c. What is the normality factor of the NaOH solution?

d. How many cubic centimeters of 0.1 *N* NaOH would be required for 100 cc. of gastric contents?

e. What is the normal value for total acidity?

f. Under what conditions would you expect high and low acidity values?

2a. How many cubic centimeters of thiosulfate solution were used in the titration?

b. What is the normality factor of the thiosulfate solution?

c. How many cubic centimeters of 0.1 *N* thiosulfate solution would be required for 100 cc. of gastric contents?

d. What is the composition of Sahli's reagent?

e. What is a normal free HCl value for gastric contents?

f. What would you expect the value to be in pernicious anemia?

3a. Is lactic acid soluble in ether?

b. What color was produced on adding $FeCl_3$?

c. Under what conditions would you expect lactic acid to be present in appreciable quantities in gastric contents?

4a. What color was produced on adding H_2O_2?

b. What would the presence of blood in gastric contents indicate?

PANCREATIC DIGESTION

Experiment 80

Trypsin, Steapsin, and Amylopsin

Prepare 3 test tubes, placing in the first tube a piece of fibrin and 5 cc. of a buffer solution[1] with a *p*H of 8.0, in the second tube 5 cc. of litmus milk,[2] and in the third tube 5 cc. of 1 per cent starch solution.[3] To each tube add 1 cc. of a 1 per cent pancreatin solution, mix thoroughly, and place in a beaker of water kept at 38°C. for 2 hours. Note any evidence of solution of the fibrin, and of change in color of the litmus milk. Test a few drops of the starch solution with Fehling's solution.

QUESTIONS

1. What is the inactive form of trypsin called?
2. What is the activator of the inactive form of trypsin?
3. What happened to the fibrin?
4. What are the end products of tryptic digestion of proteins?
5. At what *p*H does trypsin act best?
6. What happened to the litmus milk?
7. What are fat-splitting enzymes called?
8. What happened in the Fehling's test?
9. What is the end product of the action of amylopsin on starch?
10. What enzyme hydrolyzes this end product to glucose?
11. In what form are proteins absorbed?
12. In what form are fats absorbed?
13. In what form is starch absorbed?

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[1] **Buffer solution: See page 40.**

[2] **Litmus milk: See page 154.**

[3] **Starch solution: See page 108.**

BILE

Experiment 81

Tests for Important Bile Constituents

1. Test for Bile Pigments (Gmelin's Test). Place 5 cc. of concentrated HNO_3 in a test tube, and by means of a pipette add about 3 cc. of dilute bile solution carefully so that the bile solution forms a layer on the surface of the acid. Note the color produced at the juncture of the two liquids.

2. Test for Bile Salts (Pettenkofer's Test). Place 5 cc. of a dilute bile solution in a test tube, and add a few drops of 5 per cent sucrose solution. Mix thoroughly, and layer with concentrated H_2SO_4. Note the color at the juncture of the two liquids.

3. Test for Cholesterol (Liebermann-Burchard Test). Place 2 cc. of a dilute solution of cholesterol in chloroform in a dry test tube. Add 0.5 cc. of acetic anhydride and 2 drops of concentrated H_2SO_4. Note the color produced.

QUESTIONS

1a. What are the three main constituents of bile?
  b. What is the original bile pigment?
  c. What does this pigment give on oxidation and on reduction?
  d. Describe the colors produced in Gmelin's test.
2a. What are the names of the bile salts?
  b. Of what value are the bile salts in digestion?
  c. Explain the circulation of the bile salts.
  d. Describe the color produced in Pettenkofer's test.
3a. Of what are gallstones composed?
  b. Does cholesterol aid digestion?
  c. Describe the color produced in the Liebermann-Burchard test.

CALORIMETRY

Experiment 82*

Determination of the Basal Metabolic Rate

If facilities are available in a local hospital, it is suggested that the basal metabolic rate of some student be determined as a demonstration experiment for the rest of the class. The patient should have only a light supper the evening before the test is performed. He should retire early and should eat no breakfast the next morning. He should arrive at the hospital, preferably by riding, in a rested condition, and should lie down and relax for 30 minutes prior to the test. His temperature should be taken just before the test is made. A temperature above normal increases the metabolic rate 7.2 per cent for each degree Fahrenheit. A temperature below normal decreases the metabolic rate. Thus the patient's temperature, if abnormal, must be considered in interpreting the results of a metabolism test.

During the test the patient inhales pure oxygen and exhales a mixture of oxygen, carbon dioxide, and water vapor. The exhaled gases pass through soda lime, which absorbs the carbon dioxide and water vapor. Thus only unused oxygen is returned to the original supply of oxygen. As the test proceeds, oxygen is consumed; as this consumption occurs, a sloping line is drawn on a moving chart. From the slope of this line it is possible to determine the volume of oxygen consumed per minute.

These values must be corrected for temperature and the barometric pressure, which are obtained from the thermometer on the instrument and the barometer. Next, normal values for the patient are obtained from tables which furnish such values for given heights and weights, and these figures are corrected for age and sex. The basal metabolic rate of the patient is expressed in percentage plus or minus normal. For example, if the patient is found to consume 211 cc. of oxygen per minute, and if the normal rate for a person of his height and weight is 154 cc. per minute, his basal metabolic rate would be +37 per

cent. The calculations are made by dividing the number of cubic centimeters above or below normal by the normal, thus:

$$211 \text{ (actual)} - 154 \text{ (normal)} = 57$$
$$57 \div 154 = 37 \text{ per cent}$$

Most modern basal metabolism machines are equipped with instruments for simplifying calculations. By means of an adjustable T-square, which may be set for any temperature and barometric pressure, the volume of oxygen consumed, reduced to standard conditions, may be read directly by applying the T-square to the patient's chart. Then, by means of a slide rule, which may be set for volume of oxygen consumed, weight, height, age, and sex, the basal metabolic rate may be read directly. If a slide rule is used for calculating results, the answers to several of the following questions may be omitted.

QUESTIONS

1. What are the patient's weight, height, age, sex, and temperature?

2. What is the normal oxygen consumption for the patient based on weight and height?

3. What is the correction for age and sex, and what is the normal rate when this correction is applied?

4. What is the actual consumption of oxygen as read from the chart?

5. What is the temperature of the apparatus? What correction must be applied for temperature?

6. What is the barometric pressure? What correction must be made for this pressure?

7. What is the actual consumption of oxygen after corrections have been made for temperature and pressure?

8. What is the basal metabolic rate of the patient?

9. How much would 1°F. of fever raise the basal metabolic rate?

10. What effect does the ingestion of foods have on the basal metabolic rate?

11. What is this effect called?

12. What is the respiratory quotient?

13. Of what value is the respiratory quotient in nutrition studies?

14. What is the respiratory quotient during a basal metabolism test?

15. How many large Calories of heat are produced by the body during a metabolism test for each liter of oxygen consumed?

16. What disease is commonly diagnosed by means of a basal metabolism test?

17. What is meant by the gross, metabolizable, and net energy of a food?

18. How many large Calories are used by a 70-kilogram man in 24 hours during sleep and during light work?

CONNECTIVE TISSUE

Experiment 83

Composition of Bone

Place a small piece of uncooked bone in a beaker, and cover with 10 per cent HCl solution. Note any effervescence. Let stand until the next laboratory period, by which time the bone should have lost its rigidity. Remove the bone from the acid, and wash it with water. Place the bone in another beaker, add 50 cc. of water, and boil until the material goes into solution. Cool, and note the formation of a gel.

Filter the acid solution from which the bone was removed, make the filtrate alkaline with ammonium hydroxide, and finally acidify with acetic acid. Filter; dissolve the precipitate of ferric phosphate in dilute HCl. Filter again, if necessary, and test portions of the filtrate for iron with potassium ferrocyanide solution, and for phosphate with HNO_3 and ammonium molybdate solution.[1] In the test for phosphate the solution should be heated to 65°C. by placing it in a beaker of water held at that temperature.

To the acetic acid filtrate from the ferric phosphate add ammonium oxalate solution. Remove the calcium oxalate precipitated by filtration, and make the filtrate alkaline with ammonium hydroxide solution. On standing, magnesium ammonium phosphate should precipitate.

QUESTIONS

1. What happens when bone is allowed to stand in HCl solution? To what is this reaction due?
2. What is formed when the organic matter of bone is heated with water?
3. What metallic elements and what acid radicals are shown to be present in bone by the tests applied?

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[1] Ammonium molybdate solution: See page 110.

Experiment 84

Tests for Blood

1. Guaiac Test. Place about 3 cc. of a dilute solution of blood in a test tube. Acidify with a few drops of 5 per cent acetic acid, and add a 2 per cent alcoholic solution of guaiac, drop by drop, until turbidity is observed. Next add hydrogen peroxide until a color is produced.

2. Benzidine Test. To 3 cc. of a dilute solution of blood in a test tube add 3 cc. of a saturated solution of benzidine in glacial acetic acid. Add about 1 cc. of hydrogen peroxide, and note the color produced.

3. Hemin Test. Place a drop of a dilute solution of blood on a microscope slide, add a small granule of sodium chloride, and evaporate to dryness over a low flame. Add a small drop of glacial acetic acid, cover with a cover glass, and warm gently until gas bubbles form. Cool, and examine under the microscope for chocolate brown hemin crystals.

QUESTIONS

1a. What was the color produced in the guaiac test?
b. Would catalase enzyme give this test?
2a. What was the color produced in the benzidine test?
b. Would catalase enzyme give the test?
c. Will blood give this and the guaiac test after heating?
3a. How is hemin related to hemoglobin?
b. Sketch several crystals of hemin.
c. What color are hemin crystals?
d. Of these three tests for blood, which is the best for medico-legal work?

Experiment 85

Blood Plasma and Blood Serum

1. Blood Plasma. Place 5 cc. of oxalated blood[1] in a test tube, and allow the tube to stand until all the blood cells settle out. Notice the layer of plasma above the cells.

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[1] Oxalated blood for class use is best obtained from the jugular vein of a cow. A gauge 13 needle is satisfactory. A 1 liter Erlenmeyer flask, fitted

2. Blood Serum. Mix the oxalated blood used in **1** and add 5 per cent $CaCl_2$ solution, drop by drop, mixing thoroughly and allowing 3 or 4 minutes to elapse between the addition of each drop. When the blood is clotted, place the tube in a refrigerator

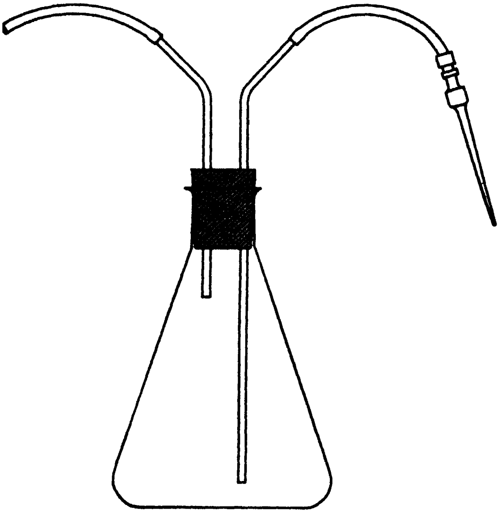

FIG. 19. Apparatus for drawing blood.

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with a two-hole rubber stopper with glass tubes passing through the holes, is a convenient receptacle for the blood. (See Fig. 19.) One glass tube should extend only through the stopper; the other should descend well into the flask. Rubber tubes should be attached to the outside ends of the glass tubes. To the rubber tube on the longer glass tube the needle is attached, the other rubber tube being used to apply suction. Suction is applied through the shorter glass tube by the mouth of the person drawing the blood. Two-tenths of a gram of powdered potassium oxalate should be present in the bottom of the flask for every 100 cc. of blood drawn.

The cow should be held securely by an assistant by means of a halter. Before introducing the needle, pressure should be applied by the left thumb of the operator to the jugular vein below the point where the needle is to be inserted. It is well to continue the pressure on the vein while the blood is being drawn. A sterile needle should be used, and the area through which the needle passes should be sterilized with alcohol. One liter of blood may be drawn from a mature cow without injury to the animal. While the blood is being drawn, the flask should be agitated to insure solution of the oxalate, thus preventing clotting. Any veterinarian should be able to supply blood obtained in this way.

for several hours or until the next laboratory period. Finally allow the tube to stand at room temperature for 1 hour, and note the appearance of blood serum.

QUESTIONS

1a. How does oxalate prevent the clotting of blood?
b. What is the appearance of blood plasma?
2a. What is the purpose of adding $CaCl_2$?
b. What is the clotting time of blood?
c. What is the appearance of blood serum?
d. How does serum differ from plasma?
e. How would you prepare blood serum from an animal?
f. State a theory explaining what happens when blood clots.

Experiment 86

Hemoglobin and Oxyhemoglobin

Note the color of the blood in the flask containing the laboratory supply. Place 5 cc. of this blood in an Erlenmeyer flask, and rotate the flask to distribute the blood in a thin film over the surface. Compare the color of this blood, which has been exposed to the air in a thin film, with the original laboratory supply. Use this blood in the following experiments.

QUESTIONS

1. Compare the original and final colors of the blood in this experiment.

2. What is the chemical difference between hemoglobin and oxyhemoglobin?

3. How is oxygen carried by blood?

4. Why is blood dark in color after being allowed to stand in large quantities?

Experiment 87

Iron in Blood

Place about 1 cc. of blood in a crucible, and heat gently over a free flame until the blood is dry. Finally ignite until only an ash remains. Cool, and dissolve the ash in a little dilute HCl. If the solution is not clear, filter into a test tube and test the solution for iron by adding a few drops of potassium ferrocyanide solution.

QUESTIONS

1. What color was produced upon the addition of potassium ferrocyanide?
2. What compound in the blood contains iron?
3. Assuming the iron in the solution to be present as $FeCl_3$, write an equation showing what happens upon the addition of $K_4Fe(CN)_6$.

Experiment 88

Hemolysis

Place 2 cc. of blood in each of 2 test tubes, and note the appearance of the blood. To the first tube add 2 cc. of 0.9 per cent NaCl solution and to the second 2 cc. of water, and mix thoroughly. Observe the 2 tubes after a few minutes.

QUESTIONS

1. What happened in each tube?
2. What is hemolysis?
3. Explain the results when 0.9 per cent NaCl was added.
4. What is the osmotic pressure, expressed in atmospheres, of red blood cells?

Experiment 89* 1 (a)

Red Blood Cell Count

[illegible]

~~Obtain from the instructor a~~ counting chamber with its cover glass and a red blood cell pipette. Make sure that the pipette is clean and dry. Carefully sterilize a fingertip and a lancet with alcohol. Do not sterilize the lancet in a flame because this practice dulls it. When the fingertip and lancet are dry, puncture the fingertip, and gently squeeze a good-sized drop of blood to the surface. Place the tip of the pipette in the drop of blood, and gently suck the blood up the stem of the pipette until it just reaches the 0.5 mark. Carefully wipe the tip of the pipette with a piece of cloth, and then place the tip in Hayem's[2] solution ~~Draw Hayem's solution~~ into the pipette until the 101 mark is reached. The work up to this point must be done rapidly to

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[2] Hayem's solution: Dissolve 0.5 gram of $HgCl_2$, 1 gram of NaCl, and 5 grams of Na_2SO_4 in 200 cc. of distilled water.

prevent clotting of the blood. If the blood clots, the pipette must be cleaned and dried, and the procedure repeated. Stretch the rubber tube around the tip of the pipette, and holding the pipette with the thumb and finger at each end, shake thoroughly for several minutes. Place the cover slip on the counting chamber, and quickly blow several drops of solution out of the pipette, discarding these drops. Next place a small drop from the pipette on the raised center of the counting chamber at the edge of the cover slip, so that the drop is drawn under the cover slip by capillary action. If the drop is too large, the liquid will flow over onto the ledge supporting the cover slip. If this occurs, rinse and dry the counting chamber and cover slip, and repeat.

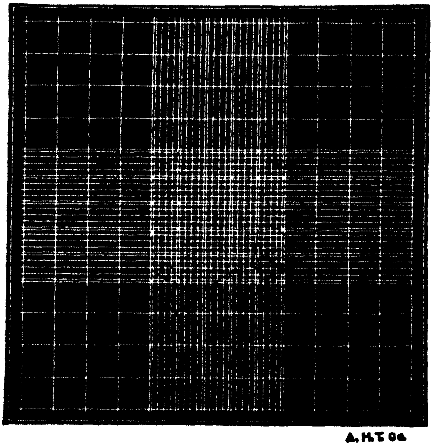

FIG. 20. Improved Neubauer ruling on a blood counting chamber.

The pipette must then be shaken again to insure a uniform suspension of cells. Place the counting chamber (see Fig. 20) under the high power of the microscope, and adjust so that the small squares in the center of the ruling are in the field of vision. Allow 2 or 3 minutes to elapse for the cells to settle; then count the cells in 5 blocks of 16 small squares. Some of the cells touch the borders of the squares. Count as in a square all the cells which touch the top and right-hand borders of a square. Do not count the cells touching the bottom or left-hand borders. When the counting is completed, the sum of the counts will be the number of cells in 80 small squares. The small squares on a counting

chamber cover an area of 1 sq. mm. Since each small square is 1/20 mm. square, there are 400 squares in a square millimeter. Eighty squares will then be 1/5 sq. mm. The depth of the counting chamber is 1/10 mm., and the dilution in the pipette is 1 to 200. Hence the number of cells in 80 squares times 5 times 10 times 200 equals the number of cells per cubic millimeter of blood. Combining these factors into a single factor, we find that the number of cells in 80 squares times 10,000 equals the number of cells per cubic millimeter of blood. When the count is complete, rinse the pipette with dilute HCl and water. Finally dry by rinsing with 95 per cent alcohol and ether. The counting chamber and cover slip should be washed with water and dried.

QUESTIONS

1. What was the number of cells in 80 small squares?
2. What is the number of cells in 1 cu. mm. of your blood?
3. What kind of counting chamber did you use?
4. What is the cost of a counting chamber?
5. What is the cost of a cover slip?
6. Why must the blood in the pipette be diluted rapidly?
7. Why is it important that the blood flow freely from the puncture?
8. Why not use the first drop of liquid from the pipette for making the count?
9. Why would it be bad technique to have the liquid spill over onto the ledge supporting the cover slip?
10. What is a normal red cell count?
11. What is the condition called where the red cell count is low?
12. Where are the red cells formed in the body?
13. What is the average life of a red blood cell?
14. What is the composition of Hayem's solution?

Experiment 90*

White Blood Cell Count

White blood cells are counted like red cells except that a different pipette is used and 1.5 per cent acetic acid serves as the diluting fluid. The acetic acid destroys the red cells and causes the white cells to stand out as black spots on the slide. Draw blood to the 0.5 mark in a white blood cell pipette, and dilute to the 11 mark with 1.5 per cent acetic acid. Mix thoroughly by shaking, discard 2 or 3 drops, and place a drop on the count-

ing chamber, allowing it to flow under the cover slip. Allow it to stand for 2 or 3 minutes for the cells to settle, and examine under the low power of the microscope. The rulings on a counting chamber (see Fig. 20) cover an area of 9 sq. mm., the central square millimeter being ruled in 400 small squares for the purpose of making a red blood cell count. The 4 corner square millimeters are ruled into 16 squares. A square millimeter just covers the microscopic field under low power. Count the number of white cells in each of the corner square millimeters, and calculate the average number of cells per square millimeter. Since the depth of the counting chamber is 1/10 mm., and since the dilution is 1 to 20, the number of cells in a square millimeter times 10 times 20 equals the number of cells per cubic millimeter of blood. Or, combining these factors, the number of cells per square millimeter times 200 equals the number of white blood cells per cubic millimeter of blood. After the count is made, clean the apparatus as described under the red blood cell count.

QUESTIONS

1. How many white blood cells were present in each of the 4 square millimeters?
2. What is the average number of white cells per square millimeter?
3. How many white blood cells are there per cubic millimeter of blood?
4. What is a normal white cell count?
5. What does a high white cell count indicate?
6. What does the diluting fluid do to the red blood cells?

Experiment 91* 2

Differential White Blood Cell Count

Place a drop of blood at one end of a thoroughly clean microscope slide, and by means of another slide draw the drop of blood across the first slide so as to make an even film. (See Fig. 21.) Allow the blood smear to dry in the air, and cover with 10 drops of Wright's stain.[3] After 1 minute add to the slide

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[3] Wright's stain: A dry, powdered Wright's stain may be obtained from the National Aniline and Chemical Company of New York. Rub up 0.1 gram of the powder in 60 cc. of special pure absolute methyl alcohol in a clean, dry mortar, until the powder has dissolved. Filter, and add 5 cc. of methyl alcohol to each 20 cc. of the filtrate.

10 drops of distilled water[4]; an iridescent scum should appear on the surface. At this point blow air on the surface through a pipette to mix the stain and water thoroughly. Allow the slide to stand for 3 minutes, pour off the stain, and dip gently in a beaker of distilled water to remove the excess stain. Wipe the water off the bottom of the slide, and allow the slide, resting on a filter paper, to drain in a vertical position. When dry, examine under the oil-immersion objective of a microscope. If the slide has been properly prepared, it should appear rose-colored when

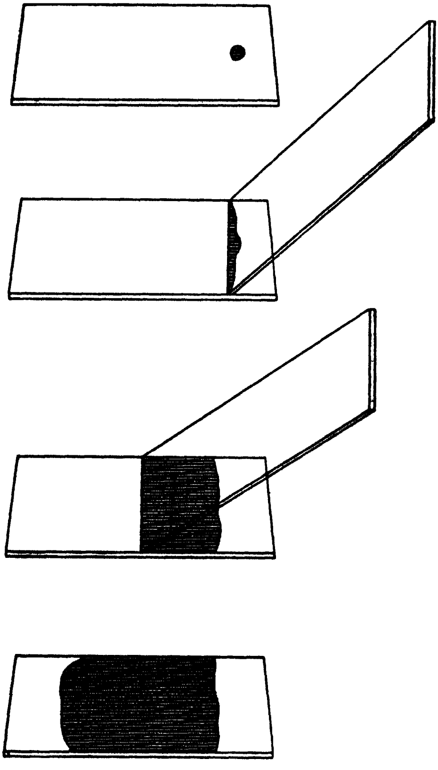

FIG. 21. Preparation of a blood smear.

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[4] A buffer solution may be used in place of distilled water for diluting Wright's stain. The buffer solution is made by dissolving 6.63 grams of monopotassium phosphate (KH_2PO_4) and 3.2 grams of disodium phosphate ($Na_2HPO_4 \cdot 12H_2O$) in 1000 cc. of distilled water.

held up to the light. Observe the appearance of the red cells, the blood platelets, and the various kinds of white cells. Cruise over the slide until you have counted 100 white cells, making note of the number of the various kinds. The values obtained represent the percentage of the various kinds of white cells present. The kinds of white cells found in normal blood are polymorphonuclears, small lymphocytes, large lymphocytes, large mononuclears, transitionals, eosinophiles, and basophiles. (See Fig. 22.)

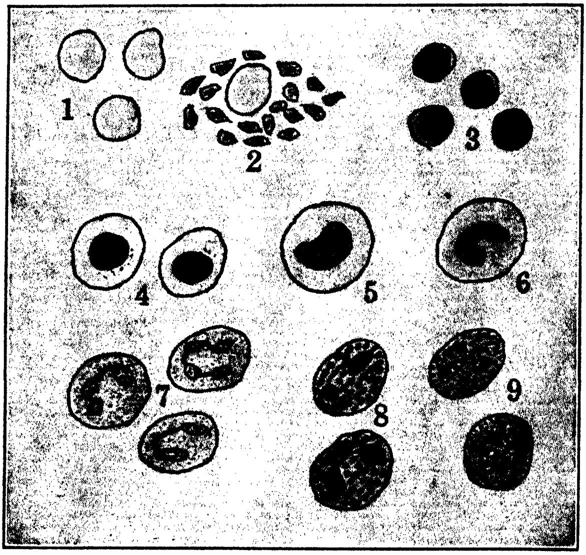

Fig. 22. Cells found in normal blood: (1) erythrocytes; (2) erythrocyte surrounded by blood platelets; (3) small lymphocytes; (4) large lymphocytes; (5) large mononuclear; (6) transitional; (7) polymorphonuclears; (8) eosinophiles — the stippling is red; (9) basophiles or mast cells — the stippling is blue.

QUESTIONS

1. Make a sketch of a red blood cell, a group of blood platelets, and each of the different kinds of white blood cells.
2. What is a normal differential white cell count?
3. What was your differential white cell count?
4. What does a high percentage of polymorphonuclears indicate?
5. What two dyes are present in Wright's stain?

Experiment 92

Determination of Hemoglobin by the Tallquist Method

Sterilize a lancet and finger with alcohol, and when both are dry, puncture the finger. Allow one large drop of blood to fall near one end of a piece of Tallquist paper, and fold the other end of the paper under the drop of blood to act as a blotter. As soon as the drop of blood has lost its sheen, place the paper under the standard hemoglobin scale so that the spot of blood covers the hole in the standard scale. Estimate the percentage of normal hemoglobin in your blood.

QUESTIONS

1. What amount of hemoglobin is present in your blood, expressed as a percentage of normal?
2. What is the actual percentage of hemoglobin in normal blood, according to Tallquist?
3. What is the actual percentage of hemoglobin in your blood?
4. The color index of blood is obtained by dividing the percentage of normal hemoglobin by the percentage of normal red cell count, considering a 5,000,000 count as normal. What is the color index of your blood?

Experiment 93*

Determination of Hemoglobin by the Newcomer Acid Hematin Method

Puncture a finger, and draw blood to the 10 mark on the Newcomer pipette. Wipe the blood from the end of the pipette, and draw HCl solution (1 cc. concentrated HCl diluted to 100 cc. with distilled water) to the mark above the bulb of the pipette. Transfer to a dry test tube, and allow to stand for 25 minutes. Place the brown light filter under the left colorimeter cup and the blue light filter in the eyepiece of the colorimeter. Place distilled water in the left colorimeter cup and the sample in the right one. Set the left plunger at a depth approximating that of the right plunger when the color comparison is made. Set the colorimeter so that the colors match, and observe the reading of the right plunger. On the brown light filter plate is a factor which, divided by the colorimeter reading in millimeters, times the dilution of the blood in the pipette, equals the grams of hemoglobin per 100 cc. of blood. The dilution in the method

described above is 1:501. This result divided by 13.8, if the Haldane scale for normal hemoglobin value is used, times 100 equals the percentage of normal hemoglobin of the sample of blood.

QUESTIONS

1. What was the reading of the colorimeter?
2. What is the factor of the brown light filter?
3. What is the dilution of the blood?
4. How many grams of hemoglobin are there per 100 cc. of blood?
5. What is the percentage of normal hemoglobin on the Haldane scale?
6. What is the percentage of normal hemoglobin on the Tallquist scale, as determined by this method? According to Tallquist, normal blood contains 15.8 grams of hemoglobin per 100 cc.
7. What is the percentage of normal hemoglobin on the Newcomer scale? According to Newcomer, normal blood contains 16.92 grams of hemoglobin per 100 cc.

Experiment 94*

Determination of Glucose in Blood by the Method of Folin and Wu

By means of pipettes transfer 5 cc. of oxalated blood and 35 cc. of distilled water to an Erlenmeyer flask. Add 5 cc. of 10 per cent sodium tungstate solution and finally 5 cc. of exactly 0.66 *N* H_2SO_4 solution. Add the H_2SO_4 solution slowly with constant agitation of the flask. Allow the mixture to stand for 20 minutes, and filter through a dry filter. Save this filtrate for Experiment 95. Transfer 2 cc. of the filtrate to a Folin-Wu sugar tube, and place in another sugar tube 2 cc. of standard glucose solution.[5] Add to each tube 2 cc. of alkaline copper solution,[6] and place the tubes in a beaker of boiling water for 8 minutes. Cool, and add to each tube 2 cc. of phosphomolybdic acid solution.[7] After 1 minute make up to the mark with water.

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[5] Glucose standard: Make a stock solution by dissolving 1 gram of pure glucose in saturated benzoic acid solution to make a volume of 100 cc. Dilute 1 cc. of this stock solution to a volume of 100 cc. with a saturated solution of benzoic acid to make the working standard, which contains 0.2 mg. of glucose per 2 cc. The working standard keeps indefinitely.

[6] Alkaline copper solution: See page 140.

[7] Phosphomolybdic acid solution: See page 142.

Mix thoroughly, and compare colors in a colorimeter, setting the standard at 20 mm. The number of milligrams of glucose per 100 cc. of blood may be calculated by substituting in the following formula:

$$\frac{\text{Reading of standard}}{\text{Reading of unknown}} \times 100 = \begin{array}{l}\text{Milligrams of glucose}\\ \text{per 100 cc. of blood}\end{array}$$

QUESTIONS

1. What is formed when sodium tungstate and H_2SO_4 are mixed?
2. In what form are the blood proteins precipitated?
3. What is the precipitate in the sugar tube after heating?
4. Explain the origin of the blue color.
5. What is the purpose of the benzoic acid in the sugar standard?
6. What is your colorimeter reading?
7. How many milligrams of glucose are present per 100 cc. of blood?
8. What is the normal blood sugar value?
9. At what concentration of sugar in the blood will sugar appear in the urine?
10. What is meant by the sugar threshold?
11. In what disease are blood sugar values high?
12. When should a blood sample for a blood sugar determination be taken in relation to a meal?
13. Why should a blood sugar determination be made soon after the blood is drawn?
14. Why is a blood sugar tube constricted?

Experiment 95*

Determination of Nonprotein Nitrogen in Blood by the Method of Folin and Wu

Transfer 5 cc. of the blood filtrate prepared in Experiment 94 to a dry Pyrex test tube graduated at 35 and 50 cc. (See Fig. 23.) Add 1 cc. of acid digestion mixture,[8] and heat over a small flame, holding the tube in a test-tube holder and shaking during the heating to prevent bumping. It is best to hold the tube in a slanting position and to heat the side of the tube just below the surface of the liquid. As heating proceeds, the solution

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[8] Acid digestion mixture: Add 50 cc. of 5 per cent copper sulfate solution to 300 cc. of 85 per cent phosphoric acid, and mix. Add 100 cc. of ammonia-free concentrated H_2SO_4. Dilute this solution with an equal volume of distilled water. Keep this solution in a glass-stoppered bottle.

is concentrated, dense white fumes appear, and the solution turns dark brown. Continue the heating, with the flame under the liquid, until the solution grows clear, loses its tan color, and becomes greenish blue. Too much heating at this point will produce a white precipitate, which interferes with the color comparison later on. Should a white precipitate form at this point, it may dissolve upon the addition of Nessler's solution. If it still persists, the determination may be saved by centrifuging just before the color comparison is made. When digestion is complete, cool, and add small quantities of distilled water, shaking after the addition of each portion of water to obtain a uniform solution. Finally cool to room temperature, and add water to the 35-cc. mark.

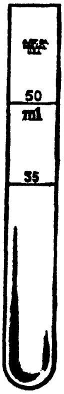

Fig. 23. Nonprotein nitrogen tube.

To prepare the standard place 2 cc. of acid digestion mixture and 3 cc. of standard $(NH_4)_2SO_4$ solution[9] in a 100-cc. volumetric flask and add about 60 cc. of distilled water. Next add 30 cc. of Nessler's solution[10] to the standard and 15 cc. to the sample in the test tube. Add water to the standard to the mark. Stopper the test tube and flask with clean rubber stoppers, and mix thoroughly. Compare colors in a colorimeter, setting the standard at 20 mm. The number of milligrams of nonprotein

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[9] Standard $(NH_4)_2SO_4$ solution: Dissolve 0.4716 gram of pure pyridine-free $(NH_4)_2SO_4$ in ammonia-free distilled water to make 1000 cc.

[10] Nessler's solution: Place 150 grams of KI, 110 grams of iodine, 100 cc. of water, and 150 grams of metallic mercury in a 500-cc. flask. Shake continuously for 7 to 15 minutes, or until the dissolved iodine has nearly all disappeared. When the iodine solution begins to turn pale, cool in running water, and continue shaking until the solution becomes greenish in color. Decant the solution into a 2-liter flask, and wash the mercury with liberal quantities of distilled water, adding the washings to the flask. Dilute with water to the mark. This solution contains the double iodide of mercury and potassium $(HgI_2{\cdot}2KI)$ and is a stock solution from which Nessler's solution is made.

For the preparation of Nessler's solution a 2.5 *N* solution of NaOH which is free from Na_2CO_3 is required. This is best prepared by making a saturated solution of NaOH and allowing it to stand in a tall, stoppered bottle until the Na_2CO_3 settles out. The clear supernatant liquid is used for making the 2.5 *N* NaOH solution. A saturated solution of NaOH

nitrogen per 100 cc. of blood may be calculated by substituting in the following formula:

$$\frac{\text{Reading of the standard}}{\text{Reading of the unknown}} \times 30 = \text{Milligrams of nonprotein nitrogen per 100 cc. of blood}$$

QUESTIONS

1. What do you understand by the term nonprotein nitrogen?
2. What was the reading of the colorimeter?
3. How many milligrams of nonprotein nitrogen were present per 100 cc. of blood?
4. What are normal values for nonprotein nitrogen?
5. Will the nonprotein nitrogen value be high or low in nephritis?
6. What is the composition of the acid digestion mixture?
7. What happens during digestion?
8. Outline briefly a method for making Nessler's solution.
9. With what compound does Nessler's solution give a color?
10. What is the composition of the standard solution?
11. Why is the acid digestion mixture added to the standard?
12. Why do you use 2 cc. of acid digestion mixture in the standard?

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contains about 55 grams of NaOH per 100 cc. of solution. Prepare 4 liters of approximately 2.5 *N* NaOH solution by mixing 730 cc. of saturated NaOH solution with enough water to make 4 liters. This should be adjusted to 2.5 *N* after titrating against a standard acid solution. To prepare Nessler's solution mix 3500 cc. of 2.5 *N* NaOH solution, 750 cc. of double iodide solution, and 750 cc. of distilled water. If properly prepared, 20 cc. of normal acid should require 11 to 11.5 cc. of Nessler's solution for neutralization, using phenolphthalein as an indicator. Also 1 cc. of acid digestion mixture should require 9 to 9.3 cc. of Nessler's solution for neutralization.

URINE

Experiment 96

Normal Constituents of Urine

1. Urea. To 10 cc. of urine in a test tube add a powdered urease tablet. Mix thoroughly, and after 10 minutes add a few drops of 50 per cent NaOH solution, and heat to boiling, holding a piece of moist red litmus paper in the fumes coming from the test tube.

2. Uric Acid. a. Isolation from Urine. Place 100 cc. of filtered urine in a beaker, and add 5 cc. of concentrated HCl. Mix thoroughly, and allow to stand until the next laboratory period. Transfer a few of the crystals in the bottom of the beaker to a microscope slide, and examine under the microscope.

b. Murexide Test for Uric Acid. Place a small amount of uric acid from the laboratory supply in an evaporating dish, add 2 or 3 drops of concentrated HNO_3, and evaporate to dryness on the steam bath. Cool, and add a drop of dilute NH_4OH.

3. Creatinine. Place 5 cc. of urine in a test tube, and add 1 cc. of saturated picric acid solution. Make the solution alkaline by adding 10 per cent NaOH solution.

4. Sulfates. Place 10 cc. of urine in a test tube, acidify with HCl, heat to boiling, and add a few drops of 10 per cent $BaCl_2$ solution.

5. Chlorides. Place 10 cc. of urine in a test tube, acidify with HNO_3, and add a few drops of $AgNO_3$ solution.

6. Phosphates. Place 10 cc. of urine in a test tube, and acidify with HNO_3. Add 2 cc. of ammonium molybdate solution,[1] and place in a beaker of water kept at 65°C.

QUESTIONS

1a. What is urease?

b. Write an equation representing what happens when urease acts on urea.

c. What happens when the litmus paper is held in the fumes? Why?

---

[1] Ammonium molybdate solution: see page 110.

d. What normal constituent of urine will give the same test without the use of urease?

e. What percentage of the total nitrogen in normal urine is in the form of urea?

f. What weight of urea is normally present in a 24-hour sample of urine?

2a. Write the formula for uric acid.

b. From what does uric acid in the human urine originate?

c. Is pure uric acid colored? Explain the origin of the color of the crystals found in your urine.

d. Describe the color changes in the murexide test.

e. What weight of uric acid is normally present in a 24-hour sample of urine?

3a. Write the formulas for creatine and creatinine.

b. What color was produced in this test?

4a. What is the main origin of sulfur in the urine?

b. Write an equation showing what happens when $BaCl_2$ is added to urine.

5a. Write an equation showing what happens when $AgNO_3$ is added to urine.

b. What weight of chloride, expressed as NaCl, is present in a 24-hour sample of urine?

6a. What happened in this test?

b. How are the phosphates in the urine related to the regulation of the *p*H of the blood?

c. What weight of phosphates, expressed as P_2O_5, is present in a 24-hour sample of urine?

Experiment 97

Pathological Constituents of the Urine[2]

1. Glucose. Mix ½-cc. portions of Fehling's solutions A and B in a test tube, and add 4 cc. of water. Heat to boiling to make sure that the Fehling's solution itself does not form a precipitate on heating. If no precipitate forms, the solution is good. Next add urine containing glucose, a few drops at a time, and heat to boiling after each addition. The total amount of urine added should not be more than 1 cc. Note the appearance of the precipitate.

2. Albumin. Place 10 cc. of clear urine containing albumin, filtered if necessary, in a Pyrex test tube, and heat the upper layer of the liquid to boiling. Add a few drops of 5 per cent

---

[2] Pathological urine: A sample of urine for these tests may be prepared by adding glucose, egg albumin, blood, and acetone to normal urine. Some indican is present in most urines.

acetic acid, and heat again. A precipitate which is insoluble in dilute acetic acid is a positive test for albumin. A precipitate formed on heating before acidification may be phosphates. They will dissolve upon the addition of acetic acid.

3. Blood. Place 2 cc. of urine containing a small amount of blood in a test tube; add 3 cc. of a fresh, saturated solution of benzidine in glacial acetic acid, and mix thoroughly. Add 1 cc. of 3 per cent hydrogen peroxide, and note the color produced.

4. Acetone (Legal's Test). Place a small amount (one crystal) of sodium nitroprusside in a test tube, add a few drops of water, and dissolve the crystal. Add 5 cc. of urine containing acetone, make alkaline with 10 per cent KOH solution, and acidify with glacial acetic acid. Note the color changes. A color on the addition of KOH is normal because of creatinine; an intensification of the color on acidifying is a positive test for acetone.

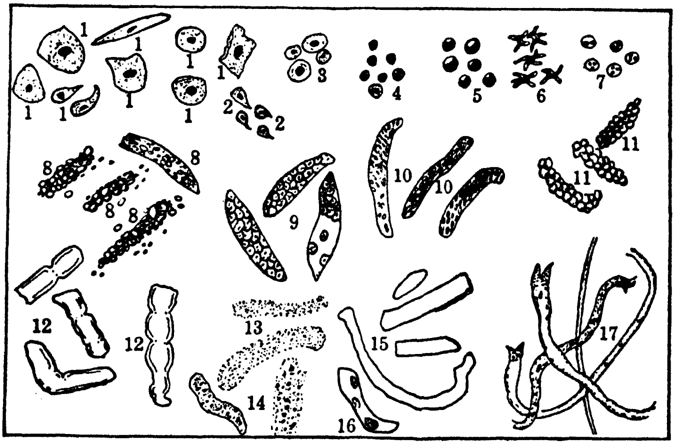

FIG. 24. Cells and casts found in urinary sediment: (1) squamous epithelium from the bladder; (2) superficial pelvic cells; (3) cells from neck of bladder; (4) renal cells; (5) pus cells (normal); (6) pus cells (ameboid form); (7) pus cells (nuclei made visible with acetic acid); (8) fatty casts; (9) epithelial casts; (10) blood casts; (11) pus casts; (12) waxy casts; (13) finely granular casts; (14) coarsely granular casts; (15) hyaline casts; (16) hyaline cast containing renal cells; (17) cylindroids.

5. Indican (Obermayer's Test). Place in a test tube 5 cc. of urine containing indican, and add an equal volume of Ober-

mayer's reagent.[3] Mix thoroughly, and add 2 cc. of chloroform. Stopper the tube, and shake. Allow the chloroform layer to settle out, and observe the color.

6. Microscopic Examination. Fill a centrifuge tube with urine, and centrifuge for 5 minutes. Carefully pour off all but a few drops of the clear liquid. Thoroughly mix the sediment in the bottom of the tube with the remaining drops of liquid, transfer to a microscope slide, and cover with a cover slip. Examine under the low and high power of the microscope, and identify as many things as possible. (See Fig. 24.) Note especially the presence of casts, pus, red blood cells, various cells, and crystals. Identify as many types of casts, cells, and crystals as possible.

QUESTIONS

1a. Describe the results of the test for glucose.

b. What normal constituent of urine may reduce Fehling's solution?

c. Why should Fehling's solution be heated before urine is added to it?

2a. What happened on heating?

b. Why is it necessary to acidify with acetic acid before identifying the precipitate as albumin?

c. What pathological condition is indicated by the presence of albumin in the urine?

3a. Describe the results of the test for blood.

b. What does blood in the urine indicate?

4a. What happened upon the addition of KOH?

b. Name two compounds which may cause this color.

c. What happened upon the addition of acetic acid?

d. What would have happened upon the addition of acetic acid if no acetone had been present?

e. What pathological condition is indicated by the presence of acetone in the urine?

f. Name two other compounds which are usually associated with acetone in the urine.

5a. What is the composition of Obermayer's solution?

b. What was the final color of the chloroform layer?

c. From what amino acid is indican derived?

d. What is the formula for indican?

e. What is the significance of large quantities of indican in the urine?

f. Are small quantities of indican in the urine considered pathological?

---

[3] Obermayer's solution: Dissolve 3 grams of ferric chloride in 1000 cc. of concentrated HCl.

6a. What did you identify in the urinary sediment?
b. What are casts? How are they formed?
c. What does the presence of casts in the urine indicate?
d. What does pus in the urine indicate?

Experiment 98*

Benedict's Quantitative Method for the Determination of Glucose in Urine

By means of a pipette transfer 25 cc. of Benedict's quantitative reagent[4] to a porcelain evaporating dish, add about 10 grams of anhydrous Na_2CO_3 and some pumice, and heat until the Na_2CO_3 has dissolved. Fill a burette with the sample of urine to be tested, and add the urine to the boiling Benedict's solution until the blue color just disappears. Add the urine fairly rapidly at first, but as the end point is approached, add it drop by drop. If the urine sample contains much glucose, dilute it 1:10 with water before making the titration, and allow for the dilution in the calculations. Since 25 cc. of Benedict's quantitative reagent requires 0.050 gram of glucose for complete reduction, there must have been 0.050 gram of glucose in the quantity of urine used for titration. Calculate the number of grams of glucose in 100 cc. of urine.

QUESTIONS

1. What is the composition of Benedict's quantitative reagent?
2. How does this differ from Benedict's qualitative reagent?
3. What compound of copper is produced when the copper is reduced by sugar?
4. What weight of glucose is required for the complete reduction of 25 cc. of Benedict's quantitative reagent?
5. How many cubic centimeters of the urine sample were required to reduce 25 cc. of Benedict's reagent?
6. What weight of glucose is present in 1 cc. of the urine sample?
7. What weight of glucose is present in 100 cc. of the urine sample?

---

[4] Benedict's quantitative reagent: Dissolve 200 grams of crystalline Na_2CO_3, 200 grams of sodium citrate, and 125 grams of KCNS in 700 cc. of distilled water, and transfer to a liter flask, filtering if necessary. Accurately weigh exactly 18 grams of crystalline copper sulfate, and dissolve in 100 cc. of water. Slowly add the $CuSO_4$ solution to the other solution, stirring constantly. Transfer all the $CuSO_4$ solution by rinsing with several portions of water. Add to this solution 5 cc. of a 5 per cent solution of $K_4Fe(CN)_6$, and make up to a volume of 1 liter.

Experiment 99

Routine Examination of a Twenty-four-hour Urine Sample

1. Collection of the Sample. Obtain a 2-liter bottle from the stockroom, and place in it 10 cc. of toluene as a preservative. Since a 24-hour sample is the urine produced by the kidneys in that length of time, it is necessary that the bladder be empty at the beginning and at the end of the collection period. If you desire to start the collection at 8 A.M., for example, the bladder should be emptied at exactly that time and the urine discarded. All subsequent urine for 24 hours should be placed in the bottle. After each addition of urine the bottle should be shaken to mix the urine with the toluene. The sample should be kept in a cool place. At exactly 8 A.M. the next morning the bladder should be emptied again, the urine being added to that in the bottle. The sample should be collected so that the end of the period comes a short time before the beginning of the laboratory period at which the sample will be examined.

2. Volume. Measure the volume of the 24-hour sample in a large graduate.

3. Specific Gravity. By means of a urinometer (see Fig. 25) determine the specific gravity of your sample. It is best to adjust the temperature of the urine to that indicated on the urinometer, which is the temperature at which the urinometer was calibrated. If readings are taken at other temperatures, add 0.001 for each 3°C. above the calibration temperature, or subtract 0.001 for each 3°C. below the temperature. When readings are taken, there should be no foam on the surface of the urine, and the urinometer should not touch the side of the cylinder.

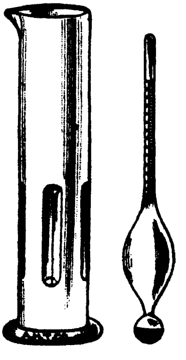

FIG. 25. Urinometer.

4. Appearance. Note the color, transparency, and odor of the sample.

5. Reaction. Test the reaction of the sample with phenolphthalein, litmus paper, and Congo red. Estimate the approximate *p*H of the sample.

6. Pathological Constituents. Test the sample for the following constituents as indicated in Experiment 97: sugar, albumin, acetone, indican, and blood.

7. Microscopic Examination. Examine the sediment from a sample of the urine under the microscope, noting the various types of sediment as indicated in Experiment 97.

QUESTIONS

1. Why is it necessary to examine a 24-hour sample of urine?

2a. What is the volume of your 24-hour sample?

b. What is a normal volume?

c. What would you expect the volume to be in diabetes? In severe nephritis?

3a. What is the specific gravity of your sample?

b. What is the normal range for specific gravity?

c. What would you expect the specific gravity to be in diabetes? In severe nephritis?

d. What is the weight of total solids in your urine as calculated by means of Long's coefficient?

4a. What was the appearance of your sample?

b. What causes cloudy urine?

c. To what is the color of urine due?

d. What foods cause urine to have an abnormal odor?

5a. What colors did your sample give with phenolphthalein, litmus, and Congo red?

b. What is the approximate *p*H of your sample?

c. What types of food produce an acid urine? An alkaline urine?

6a. What pathological constituents were present in your sample?

b. Is indican in small quantities considered pathological?

7a. Name the various things you observed in the microscopic examination.

b. Are any of these things considered pathological?

APPENDIX

For general laboratory use the following desk reagents are suggested:

Concentrated NH_4OH.
Concentrated HCl.
Dilute HCl (1 part concentrated HCl to 1 part H_2O).
Concentrated HNO_3.
Dilute HNO_3 (1 part concentrated HNO_3 to 2 parts H_2O).
Concentrated H_2SO_4.
Dilute H_2SO_4 (1 part concentrated H_2SO_4 to 4 parts H_2O).

The following is a list of special apparatus necessary for the performance of the experiments in this book:

Analytical balance.
Analytical weights.
Basal metabolism apparatus.
Blood-letting lancet.
Centrifuge.
Centrifuge tubes.
Colorimeter.
Desiccator.
Einhorn saccharometers.
Ether extraction apparatus.
Folin-Wu nonprotein nitrogen tubes.
Folin-Wu sugar tubes.
Hemacytometer.
Kjeldahl apparatus and flasks.
Lactometer.
Microscope.
Microscope slides and cover slips.
Needles, gauge 13, for drawing blood from cows.
Newcomer standard glass plates and pipette.
Nichrome or platinum wires.
Pipettes, 1, 2, 3, 5, 10, 25, and 50 cc.
Polariscope or saccharimeter.
Porcelain spot plates.
Soda lime tubes, medium size.
Tallquist hemoglobin scale.
Urinometer.
Volumetric flasks, 100, 500, 1000, and 2000 cc.

The following is a list of chemicals needed to perform the experiments in this book, with suggested quantities which should be ample for a class of twenty-five students:

	POUNDS	OUNCES	
Acetic anhydride	..	4	..
Acetone	10	..	..
Acid, acetic, glacial	10	..	..
Benzoic	..	4	..
Boric	2	..	..
Hydrochloric, concentrated	20	..	..
Lactic	2	..	..
Molybdic	1	..	..
Nitric concentrated	15	..	..
Phosphoric, 85 per cent	2	..	..

	Pounds	Ounces	
Picric	1	..	..
Salicylic	..	4	..
Sulfanilic	..	1	..
Sulfuric, concentrated	30	..	..
Sulfuric, concentrated, ammonia-free	10	..	..
Trichloracetic	..	8	..
Uric	..	4	..
Agar-agar	2	..	..
Albumin, egg, dried	2	..	..
			Gallons
Alcohol, ethyl	..	..	10
Methyl, pure	1	..	..
Ammonium hydroxide	10	..	..
Ammonium oxalate	..	8	..
Ammonium sulfate	10	..	..
Ammonium sulfate, pyridine-free	..	1	..
Aniline	..	2	..
Barium hydroxide	2	..	..
Benzidine	..	1	..
Bile, ox, dried	..	4	..
Bismuth subnitrate	..	8	..
Bone black	2	..	..
Borax	..	4	..
Bromine	..	4	..
Bromothymol blue	..	1	..
Calcium chloride	1	..	..
Camphor	..	1	..
Chloroform	5	..	..
Cholesterol	..	1	..
Collodion	5	..	..
Congo red	..	1	..
Copper turnings	1	..	..
Cotton	1	..	..
Cupric acetate	1	..	..
Cupric chloride	..	4	..
Cupric sulfate	5	..	..
Dextrin	1	..	..
p-Dimethylaminobenzaldehyde	..	1	..
Dipotassium phosphate	2	..	..
Disodium phosphate	1	..	..
Ether, ethyl	10	..	..
Petroleum	5	..	..
Ferric chloride	..	8	..
Fibrin	..	8	..
Flour, hard spring wheat	5	..	..
Formalin	1	..	..
Fructose	..	8	..

	Pounds	Ounces	
Galactose	1	..	..
Gelatin	1	..	..
Glucose	5	..	..
Glycerol	..	8	..
			Grams
Gold chloride	..	..	5
Gum arabic	..	8	..
Gum guaiac	..	2	..
Hemp seed	5	..	..
Hydrogen peroxide, 3 per cent	5	..	..
Inulin	..	4	..
Iodine	1	..	..
Iron filings	1	..	..
Lactose	1	..	..
Lard	2	..	..
Lead acetate	1	..	..
			Strips
Litmus, paper, red and blue	..	..	500
Powdered	..	2	..
Magnesium, powdered	..	4	..
Magnesium sulfate	..	4	..
Maltose	1	..	..
Mannose	..	4	..
Mercuric chloride	..	4	..
Mercury	5	..	..
Methyl orange	..	1	..
Methyl red	..	1	..
Methylene blue	..	1	..
Monopotassium phosphate	2	..	..
α-Naphthol	..	1	..
			Grams
Ninhydrin	..	..	1
			Quarts
Oil, corn	..	..	1
Cottonseed	..	..	1
Linseed	..	..	1
Olive	..	..	1
Pancreatin	..	2	..
			Strips
Paper, fat-free strips	..	..	50
Paraffin wax	1	..	..
Pepsin	..	2	..
Peptone	2	..	..
Phenol	..	4	..
Phenolphthalein	..	2	..
Phenylhydrazine	..	4	..
Potassium acid phthalate	2	..	..

	POUNDS	OUNCES	
Potassium carbonate	1	..	..
Potassium dichromate	1	..	..
Potassium ferrocyanide	..	4	..
Potassium hydrogen sulfate	..	4	..
Potassium hydroxide	5	..	..
Potassium iodate	..	4	..
Potassium iodide	2	..	..
Potassium nitrate	1	..	..
Potassium oxalate	..	4	..
Potassium thiocyanate	1	..	..
Rennin	..	2	..
Resorcinol	..	1	..
Sand	5	..	..
Silver nitrate	1	..	..
Soda lime	2	..	..
Sodium alizarin sulfonate	..	1	..
Sodium bicarbonate	1	..	..
Sodium carbonate, anhydrous	5	..	..
Crystals	1	..	..
Sodium chloride	10	..	..
Sodium citrate	2	..	..
Sodium hydroxide	10	..	..
Flakes	1	..	..
For Kjeldahl	20	..	..
Sodium molybdate	1	..	..
Sodium nitrite	..	4	..
Sodium nitroprusside	..	1	..
Sodium potassium tartrate	5	..	..
Sodium sulfate	2	..	..
Sodium tartrate	..	4	..
Sodium thiosulfate	1	..	..
Sodium tungstate	2	..	..
Starch, arrowroot	..	4	..
Corn	1	..	..
Potato	..	4	..
Rice	..	4	..
Wheat	..	4	..
Sucrose	5	..	..
Toluene	..	4	..
Tyrosine	..	1	..
Urea	1	..	..
Wheat bran	2	..	..
Wright's stain	..	1	..
Xylene	..	4	..
Xylose	..	4	..
Zinc chloride	..	8	..
Zinc, mossy	1	..	..

INTERNATIONAL ATOMIC WEIGHTS

1941

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	Symbol	Atomic Number	Atomic Weight		Symbol	Atomic Number	Atomic Weight
Aluminum	Al	13	26.97	Molybdenum	Mo	42	95.95
Antimony	Sb	51	121.76	Neodymium	Nd	60	144.27
Argon	A	18	39.944	Neon	Ne	10	20.183
Arsenic	As	33	74.91	Nickel	Ni	28	58.69
Barium	Ba	56	137.36	Nitrogen	N	7	14.008
Beryllium	Be	4	9.02	Osmium	Os	76	190.2
Bismuth	Bi	83	209.00	Oxygen	O	8	16.0000
Boron	B	5	10.82	Palladium	Pd	46	106.7
Bromine	Br	35	79.916	Phosphorus	P	15	30.98
Cadmium	Cd	48	112.41	Platinum	Pt	78	195.23
Calcium	Ca	20	40.08	Potassium	K	19	39.096
Carbon	C	6	12.010	Praseodymium	Pr	59	140.92
Cerium	Ce	58	140.13	Protactinium	Pa	91	231
Cesium	Cs	55	132.91	Radium	Ra	88	226.05
Chlorine	Cl	17	35.457	Radon	Rn	86	222
Chromium	Cr	24	52.01	Rhenium	Re	75	186.31
Cobalt	Co	27	58.94	Rhodium	Rh	45	102.91
Columbium	Cb	41	92.91	Rubidium	Rb	37	85.48
Copper	Cu	29	63.57	Ruthenium	Ru	44	101.7
Dysprosium	Dy	66	162.46	Samarium	Sm	62	150.43
Erbium	Er	68	167.2	Scandium	Sc	21	45.10
Europium	Eu	63	152.0	Selenium	Se	34	78.96
Fluorine	F	9	19.00	Silicon	Si	14	28.06
Gadolinium	Gd	64	156.9	Silver	Ag	47	107.880
Gallium	Ga	31	69.72	Sodium	Na	11	22.997
Germanium	Ge	32	72.60	Strontium	Sr	38	87.63
Gold	Au	79	197.2	Sulfur	S	16	32.06
Hafnium	Hf	72	178.6	Tantalum	Ta	73	180.88
Helium	He	2	4.003	Tellurium	Te	52	127.61
Holmium	Ho	67	164.94	Terbium	Tb	65	159.2
Hydrogen	H	1	1.0080	Thallium	Tl	81	204.39
Indium	In	49	114.76	Thorium	Th	90	232.12
Iodine	I	53	126.92	Thulium	Tm	69	169.4
Iridium	Ir	77	193.1	Tin	Sn	50	118.70
Iron	Fe	26	55.85	Titanium	Ti	22	47.90
Krypton	Kr	36	83.7	Tungsten	W	74	183.92
Lanthanum	La	57	138.92	Uranium	U	92	238.07
Lead	Pb	82	207.21	Vanadium	V	23	50.95
Lithium	Li	3	6.940	Xenon	Xe	54	131.3
Lutecium	Lu	71	174.99	Ytterbium	Yb	70	173.04
Magnesium	Mg	12	24.32	Yttrium	Y	39	88.92
Manganese	Mn	25	54.93	Zinc	Zn	30	65.38
Mercury	Hg	80	200.61	Zirconium	Zr	40	91.22

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